

Microbial Commercial Activity Notice

40 C.F.R. § 725.155

to the

**U.S. Environmental Protection Agency
Office of Pollution Prevention and Toxic
Chemical Control Division
New Chemicals Notice Management Branch**

[REDACTED]

Date of Submission:

[REDACTED]

Submitter:

[REDACTED]

Submitted to:

**TSCA Document Processing Center (7407)
Room L-100
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, D.C. 20460**

SUBSTANTIATION OF CONFIDENTIALITY

The following information is claimed confidential in this submission:

1. Chemical identity (Sections 2 and 3);
2. Submitter identity (Sections 1 and 6);
3. Specific patent and patent application numbers (Section 1);
4. Proprietary assessment of published literature (Section 3);
5. Proprietary robust summaries of surrogate testing (Section 3);
6. Specific use information (Section 1);
7. Specific manufacturing volumes (Section 5);
8. Plant site and specific manufacturing description, including byproducts (Sections 4, 6, 8 and 9);
and
9. Specific processing description (Section 7 and 8).

Items 6 – 9 are exempt from substantiation per section 14(c)(2) of TSCA. Items 1 and 6 are also trade secret information, justification for which is provided below.

To accompany the electronic certification required per section 14(c)(1)(A) and (B), substantiation is given concurrent with this submission to maintain the confidential business information (CBI) status of items 1 – 5 in accordance with 40 C.F.R. § 725.94, section 14(c)(3) of TSCA and the Notice issued on January 19, 2017 (82 Fed. Reg. 6522). Portions of the substantiation that discuss these items are claimed confidential as well.

The Submitter notes the continued inconsistency between the statute and the regulations concerning when substantiation is due for item 1, the CBI claim related to chemical identity. Section 14(c)(2)(G) of TSCA does not require substantiation for chemical identity claims prior to non-exempt commercial distribution. However, the regulations still require this substantiation at the time the MCAN is submitted for agency review. Given the critical business need to maintain CBI and trade secret protection for the chemical identity of the strain, substantiation is provided for this item concurrent with the MCAN submission out of an abundance of caution.

To comply with section 14(c)(1)(C) of TSCA, the generic name for the microorganisms pursuant to 40 C.F.R. §§ 725.80(a)(1) and 725.85(a)(3)(ii) is “**Biofuel producing *Saccharomyces cerevisiae* modified, genetically stable**”). See also Section 1.3 of this MCAN. The generic category of use description pursuant to §§ 725.80(a)(2) and 725.88(b) is “**ethanol production**”. See Section 1.4 of this MCAN.

A. For what period of time is a claim of confidentiality being asserted? If the claim is to extend until a certain event or point in time, indicate that event or time period. Explain why the information should remain confidential until such point.

Based on the following, this information should be held confidential for the full 10-year period initially allowed by law, and it should remain eligible for renewal until the technology is obsolete, or until the microorganism is widely known because of competing research. This is because disclosure of the information will reveal confidential innovative contributions of the construct that would reduce the time competitors need to enter the market. Reducing the length of time that we are the exclusive provider of this innovation has a direct, adverse economic impact on our Company. More specifically, if our competitors enter the market sooner, we will lose potential or actual customers which in turn will reduce revenues currently projected from establishing and securing a long-term customer base for this product over the next ten years and beyond.

B. Briefly describe any physical or procedural restrictions within the company or institution relating to the use and storage of the information claimed as confidential. What other steps, if any, apply to use or further disclosure of the information?

Internally, chemical identity, specific patent, and proprietary assessments are treated as confidential and only those with a need-to-know have access to this information.

The information does not leave the site of R&D, production or testing in a form which is accessible to the public or its competitors. The MCAN microorganism does not leave the site of testing or production in a form which is accessible to the public or its competitors unless those competitors have executed nondisclosure agreements to protect the confidentiality of the strain as part of a mutually beneficial business venture that is planned or underway. Secure handling impedes product analysis by others.

C. Has the information claimed as confidential been disclosed to individuals outside of the company or institution? Will it be disclosed to such persons in the future? If so, what restrictions, if any, apply to use or further disclosure of the information?

The Submitter securely guards chemical identity, submitter identity, specific patent and patent application numbers, the selection and assessment of the literature and robust surrogate data summaries in association with the development of the new strain. We share the information with the EPA on a confidential basis. We also share this information with outside legal counsel engaged to provide legal services to Submitter, where such information is both confidential and protected under the attorney-

client privilege. In specific cases, we also may share information with outside third parties in strict confidence in connection with certain business transactions such as a mutually-beneficial joint venture arrangement with a foreign company or an intellectual property licensing arrangement. In those cases, the information is shared in a manner that protects its confidentiality through the use of signed nondisclosure agreements that delineate specific security measures taken to ensure that the Submitter's information is kept secure and shared on a need-to-know basis within the third-party organization (inclusive of its agents and law firms). In general, however, the release of the information could reveal sensitive information that competitors can act on about the commercial status, time-to-market, and production advantages of the MCAN strain. With respect to the robust surrogate summary information in this filing, [REDACTED]

[REDACTED] Given the extensive time and resources that were required to generate these unpublished data, the summaries are securely guarded by the Submitter to preserve the commercial advantage of owning information that is required for commercialization against competitors that have not invested the same resources to obtain data themselves. Our competitors do not know the strain is being manufactured in the absence of information shared as part of a specific mutually-beneficial commercial arrangement as described above.

D. Does the information claimed as confidential appear, or is it referred to, in any of the following questions? If the answer is yes to any of these questions, indicate where the information appears and explain why it should nonetheless be treated as confidential.

a. Advertising or promotional materials for the microorganism or the resulting end product?

No advertising or promotional material discloses the chemical identity, submitter identity, patent information, literature assessment, or the robust surrogate test summaries.

b. Material safety data sheets or other similar materials for the microorganism or the resulting end product?

No safety data sheet or similar materials disclose the chemical identity, submitter identity, patent information, literature assessment, or the robust surrogate test summaries.

c. Professional or trade publications?

No professional or trade publications disclose the chemical identity, submitter identity, patent information, literature assessment, or the robust surrogate test summaries.

d. Any other media available to the public or to competitors?

No other media available to the public or competitors discloses the chemical identity, submitter identity, patent information, literature assessment, or the robust surrogate test summaries.

e. Patents

See Section H below.

f. Local, State, or Federal agency public files?

No local, state, or federal agency public files disclose the chemical identity, submitter identity, patent information, literature assessment, or the robust surrogate test summaries.

E. Has EPA, another Federal agency, a Federal court, or a State made any confidentiality determination regarding the information claimed as confidential? If so, provide copies of such determinations.

The EPA has reviewed our confidentiality claims for similar or the same information in prior MCANs. As a result of those reviews, we have been instructed to voluntarily release certain confidentiality claims. No confidential claims are made in this submission for information that we have previously agreed to release in earlier submissions. There have been no other rulings on confidentiality for the chemical identity, submitter identity, specific patent and patent application numbers, or the selection and assessment of the literature and robust surrogate data summaries in this MCAN. No federal, local or state agency or court has public files disclosing the information claimed confidential in this MCAN.

F. For each type of information claimed confidential, describe the harm to the company's or institution's competitive position that would result if this information were disclosed. Why would this harm be substantial? How could a competitor use such information? What is the causal connection between the disclosure and harm?

The nature of the Submitter's business as a leading provider of yeast for industrial ethanol production is highly competitive. A competitor would be able to discern the innovative contributions of the construct from disclosure of the chemical identity, submitter identity, specific patent and patent application numbers, the selection of published literature, the proprietary assessment of the literature,

or methods and chemical identities in robust surrogate data summaries. The CBI information on the identity of the MCAN strain if disclosed would provide information about how we are able to achieve highly sought-after commercial advantages in yeast [REDACTED]
[REDACTED]

Similarly:

- The disclosure of Submitter identity would provide our competitors with an early signal that we are getting ready to introduce an innovation. This would allow a competitor to more effectively work against the planned launch of the product and could weaken the initial market penetration currently expected for the product.
- Disclosure of chemical identity and related patent information would permit competitors to understand the advantages of the innovation and enter the market with a competing product much more readily. Competitors would be able to circumvent their own research and development processes, saving competitors time and money, if they gained access by way of EPA disclosure to information that the Submitter has developed at considerable cost.
- There are few published summaries on the body of scientific literature concerning the parental strain. For this reason, the Submitter considers the proprietary assessment of the published literature in Section 3 of the MCAN and the robust summaries of surrogate testing to be a proprietary Submitter report. The analysis of the literature was prepared by experts in the field specifically for the Submitter, the analysis is owned by the Submitter, and it is not publicly available. The robust testing summaries represent an even more substantial investment of time, resources and interpretive analysis by the Submitter. Competitors should have to make comparable investments to gain this knowledge. A competitor, upon obtaining this information, could use it to support a competing product. Competitors would have much less of an investment in establishing the safety of their competing product to the Submitter's business detriment. Such disclosure without having strict confidentiality protection requirements and procedures in place is intolerable. It is respectfully submitted that the availability of the individual papers and EPA's own assessment allows for a clear interpretation of the overall health and safety status of the strain. The specific microorganism identity or modifications thereto are not necessary to interpret that information.

[REDACTED]

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Disclosure to the public of the foregoing CBI would allow a competitor to enter the market more easily because competitors have the facilities, personnel and expertise to produce the microorganisms.

Because the techniques for engineering the microorganisms are generally familiar, the confidentiality of information related to the Submitter's development of the specific organisms and proprietary assessments must be maintained. Unless this confidential information is disclosed, the cost to competitors to develop a strain with similar use conditions is several million dollars and three to five years.

G. If EPA disclosed to the public the information claimed as confidential, how difficult would it be for the competitor to enter the market for the resulting product? Consider such constraints as capital and marketing cost, specialized technical expertise, or unusual processes.

Disclosure to the public of the foregoing CBI would allow a competitor to enter the market more easily because competitors have the facilities, personnel and expertise to produce the microorganisms.

Because the techniques for engineering the microorganisms are generally familiar, the confidentiality of information related to the Submitter's development of the specific organisms and proprietary assessments must be maintained. Unless this confidential information is disclosed, the cost to competitors to develop a strain with similar use conditions is several million dollars and three to five years.

H. Has the microorganism or method of production been patented in the U.S. or elsewhere? If so, why is confidentiality necessary?

Disclosure of related patent information would permit competitors to understand the advantages of the innovation and enter the market with a competing product much more readily. Competitors would be able to circumvent their own research and development processes, saving competitors time and money, if they gained access by way of EPA disclosure to information that the Submitter has developed at considerable cost. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] However, the filing and eventual publication of these applications and the issuance of these patents does not defeat CBI claims for the specific chemical identity submitted herein. The patent documents disclose aspects and characteristics common to a class of microorganisms. The microorganism submitted herein is one of many microorganisms disclosed categorically via their common attributes. Moreover, the patent documents do not fully disclose all attributes of the microorganism submitted herein, in that only some aspects are described in the patent applications.

The identity and use of the microorganism submitted herein should be treated as confidential because the patent documents do not disclose the microorganism's identity and use. Furthermore, the filing and issuance of the patent documents does not indicate that this microorganism or any other microorganisms described in the patent documents are commercially available in the U.S. Although the strain is described in the patent documents, it is included within a body of information that makes it difficult for a competitor to discern the specific innovation that is being pursued by the submission of this MCAN. The patent relationship is maintained as CBI to prevent competitors from discerning the commercial status of the strain. The knowledge that this strain is the subject of an MCAN submission also is CBI. The disclosure of this information would lead to competitive harm as already described.

I. Does the microorganism leave the site of production or testing in a form which is accessible to the public or to competitors? What is the cost to a competitor, in time and money, to develop appropriate use conditions? What factors facilitate or impede product analysis?

The information claimed as CBI does not leave the site of R&D, production, or testing in a form which is accessible to the public or competitors. The MCAN microorganism does not leave the site of testing or production in a form which is accessible to the public or competitors. Secure handling impedes product analysis by others. For more information on this issue, see Section B above. Unless this confidential information is disclosed, the cost to competitors to develop a strain with similar use conditions is several million dollars and three to five years. For more information on this cost and the factors that facilitate or impede product analysis, see Section F above.

- J. For each additional type of information claimed as confidential, explain what harm would result from disclosure of each type of information if the identity of the microorganism were to remain confidential.**

See Section F above.

- K. Would the disclosure of the information claimed confidential reveal: confidential process information, or information unrelated to the effects of the microorganism on health and the environment? Describe the causal connection between the disclosure and harm.**

Yes. Disclosure of specific strain information on the modifications described in this submission will release confidential process information on [REDACTED]

[REDACTED]. This process information is separately claimed as CBI in this MCAN submission. This would allow competitors to devote fewer resources to research and development and competing for customers because they would be able to easily discern the microorganism, the process, and the commercial use. It would give competitors an advantage in knowing how to create the microorganism for the same process without the necessity of undergoing research and development to determine how best to create the microorganism. The Submitter considers as highly confidential the identity of, and the advancements achieved through, the modifications. The modifications distinguish the microorganism from more conventional strains and contribute new and useful performance properties to the microorganism. Disclosure without having the appropriate nondisclosure protections in place would impart knowledge of how the strain was created and how it enhances the ethanol production process. This would significantly reduce the commercialization time of competitors to create the microorganisms.

- L. Does the company or institution assert that disclosure of the microorganism identity is not necessary to interpret any health and safety studies which have been submitted? If so, explain how a less specific identity would be sufficient to interpret the studies.**

The Submitter, pursuant to 40 C.F.R. § 725.92(b) and § 725.95(e), claims as confidential references to microorganism identity and information that would facilitate the discovery of its identity in (1) certain information in published scientific journal articles submitted with the MCAN, and (2) proprietary literature assessments and our robust data summaries. Disclosure of specific microorganisms or modification types in these documents would help to reveal the nature of the modifications in the MCAN strain. Such disclosure would allow competitors to devote fewer resources to research and development because they would be able to more easily discern the modifications and commercial use.

Furthermore, such disclosure would give competitors an advantage by imparting direct knowledge about how to create the modifications without any effort on their part or a commensurate investment in the research and development. As noted above, the analysis of the published literature in this Submission was prepared by experts in the field specifically for the Submitter, the analysis is owned by the Submitter, and it is not publicly available. The robust testing summaries also represent a substantial investment of time, resources and interpretive analysis by the Submitter. Competitors should have to make comparable investments to gain this knowledge. A competitor, upon obtaining this information, could use it to support a competing product. It is respectfully submitted that the availability of the individual papers and EPA's own assessment allows for a clear interpretation of the overall health and safety status of the strain. The specific microorganism identity or modifications thereto are not necessary to interpret that information.

M. Does any of the information you are claiming as CBI contain (a) trade secret(s)?

Yes. The chemical identity of the microorganisms (Section 2), including the modifications made, the specific genetic sequences, the process by which the modifications are made, and the specific use constitute commercially valuable plans that are proprietary and trade secret. The applicable definition of a trade secret is "a secret, commercially valuable plan, formula, process, or device that is used for the making, preparing, compounding, or processing of trade commodities and that can be said to be the end product of either innovation or substantial effort." *Public Citizen Health Research Group v. FDA*, 704 F.2d 1280, 1288 (D.C. Cir. 1983). A direct relationship exists between the claimed trade secret information, the end product of the innovation, and the substantial effort of the Submitter to develop the innovation. The end product of the modifications is a trade commodity (yeast) that is commercially valuable because of [REDACTED]. The specific chemical identity, process and use information distinguishes the commercial product from more conventional products. There is a direct relationship between the information claimed trade secret and the ethanol production process. Its disclosure without appropriate confidentiality protections and procedures in place would reveal the Submitter's commercially valuable formula and plan to [REDACTED]

The construct directly contributes to these new and useful performance and economic properties. EPA's release of the information would let a competitor discern that the Submitter is launching a superior product that offers customers the commercial advantage of [REDACTED]

[REDACTED] The genetic construct description would impart

knowledge of how to achieve the commercially superior product that forms the basis of the Submitter commercial plans. EPA disclosure of any of this information alone or in combination would allow a competitor the time and ability to launch a competing effort that could harm the commercial return from the Submitter's investment. Maintaining this information as trade secret is required to reduce the likelihood of a competitor manufacturing a similar product without investing time in conducting the necessary research and development required to develop such a product.

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1.1 Purpose

[REDACTED] during ethanol production. The modified *S. cerevisiae* strain is intended to act as a replacement for the standard yeast with the intention of reducing or eliminating [REDACTED]

In accordance with 40 C.F.R. § 725.155(c), the following information is provided.

[REDACTED]

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Submitter: [REDACTED]

Address: [REDACTED]

Contact: [REDACTED]
[REDACTED]

Technical Contact: [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

An agent letter is provided as **Attachment 1**. The certification statement is provided in **Attachment 2**.

1.3 Proposed Generic Name

The explicit biological name of the microorganism is *Saccharomyces cerevisiae* strain

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] A

structurally representative generic name for the microorganisms that is in accord with 40 C.F.R. § 725.85 and page 56 of EPA's June 2, 1997 Points to Consider guidance document is "***Biofuel producing Saccharomyces cerevisiae modified, genetically stable.***"

This generic name protects the confidential identity of [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]. The Submitter considers the identity of the genes used to modify the

microorganism as highly confidential, *i.e.*, [REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED] Nondisclosure of the specific modifications is required to reduce the likelihood of a competitor manufacturing a similar product without investing time and expense in conducting the necessary research and development required to develop such a product.

1.4 Proposed Use Category and Generic Use Description

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED] The Submitter proposes the following generic use description: “ethanol production.” This description protects the confidential process and purpose of the MCAN strain from disclosure.

2. Microorganism Identity Information

In accordance with 40 C.F.R. § 725.155(d), the following information is provided. [REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

2.1 Recipient Strain Identification

[REDACTED]

Taxonomic characteristics are the following:

Name: *Saccharomyces cerevisiae*

Class: *Saccharomyces*

Order: *Saccharomycetales*

Genus: *Saccharomyces*

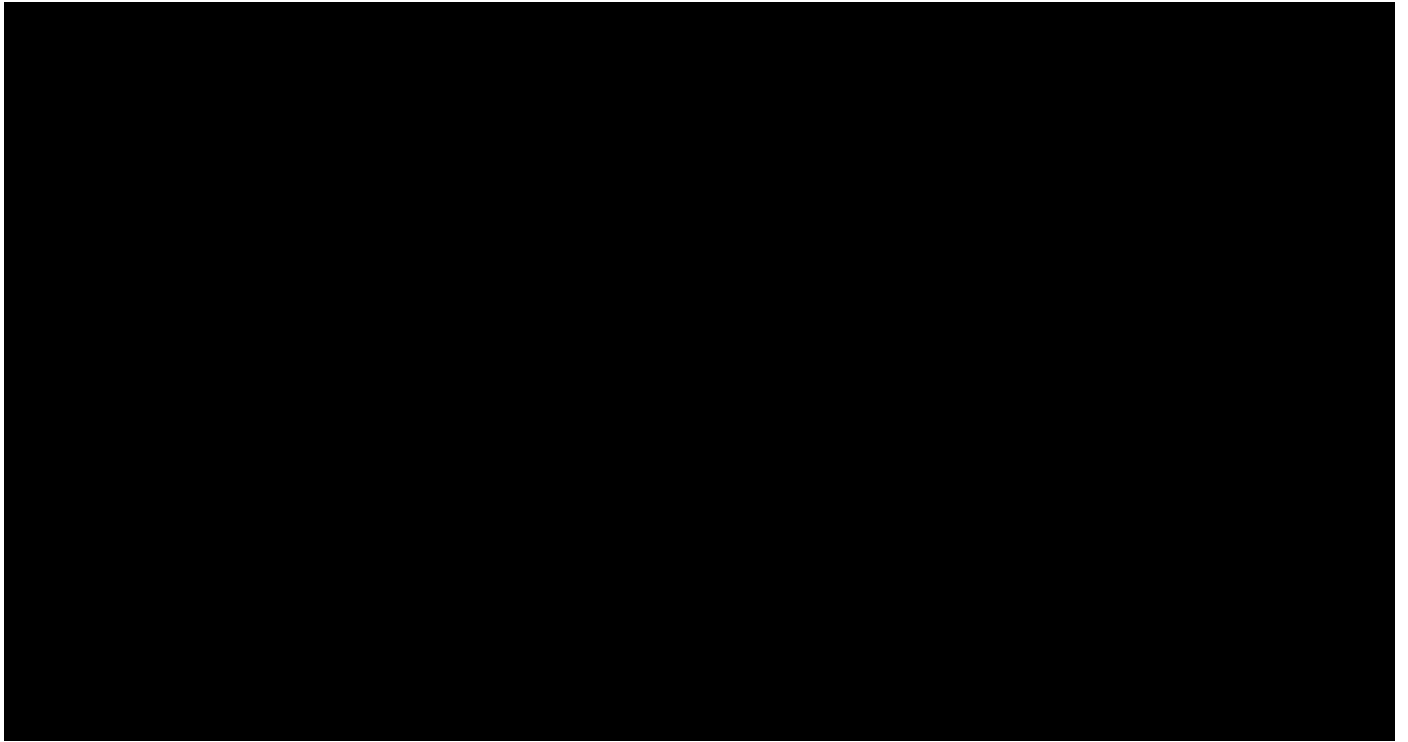
Species: *cerevisiae*

[REDACTED]

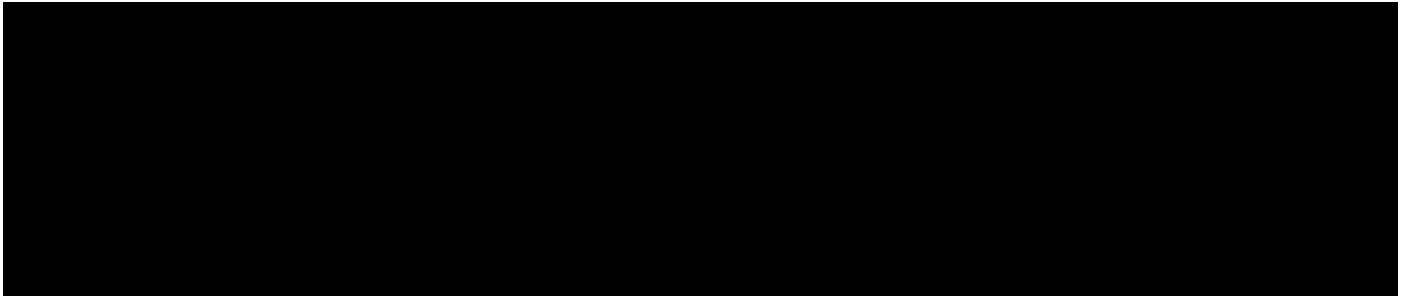
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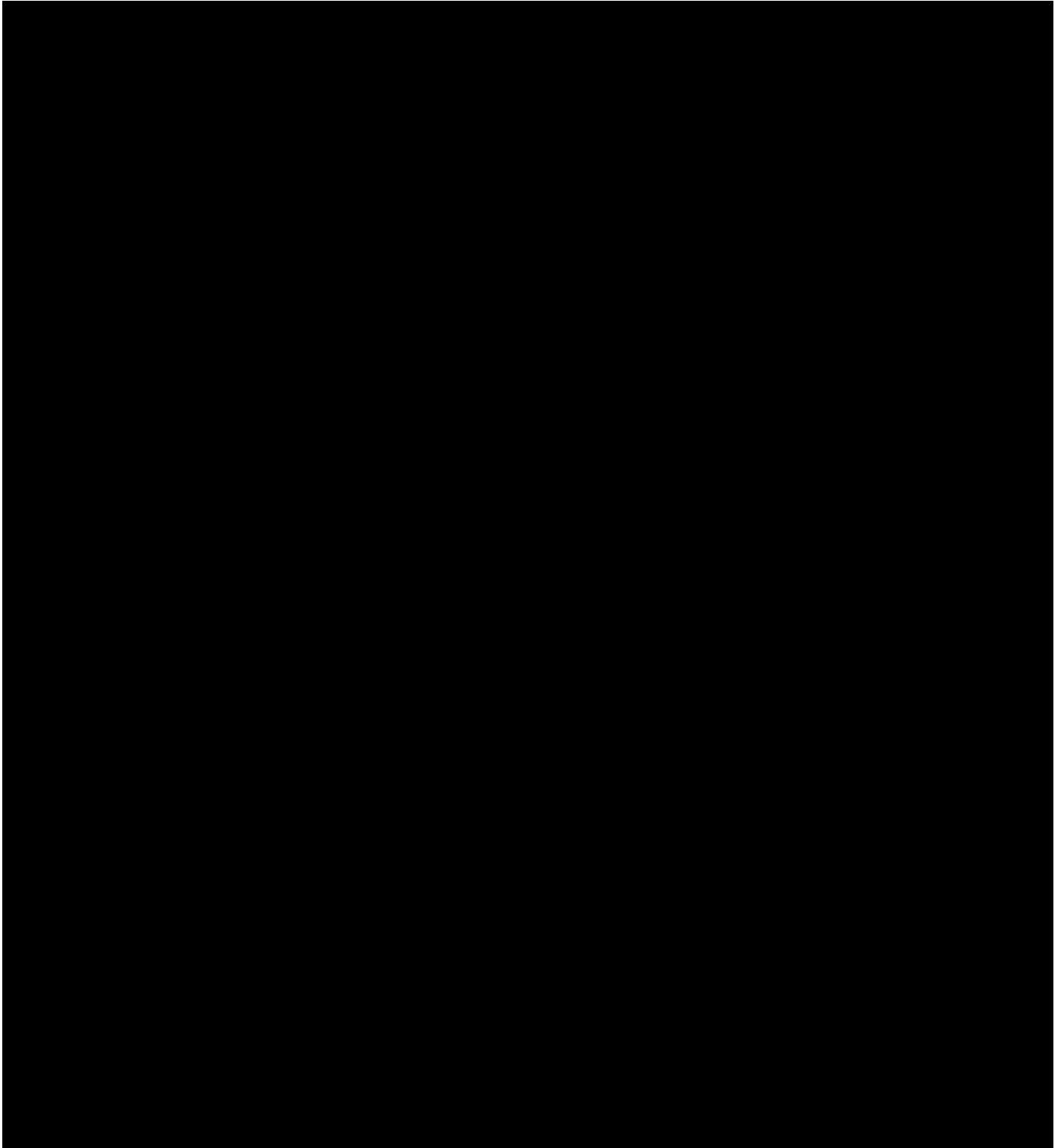
2.2 Modified Strain



[REDACTED]

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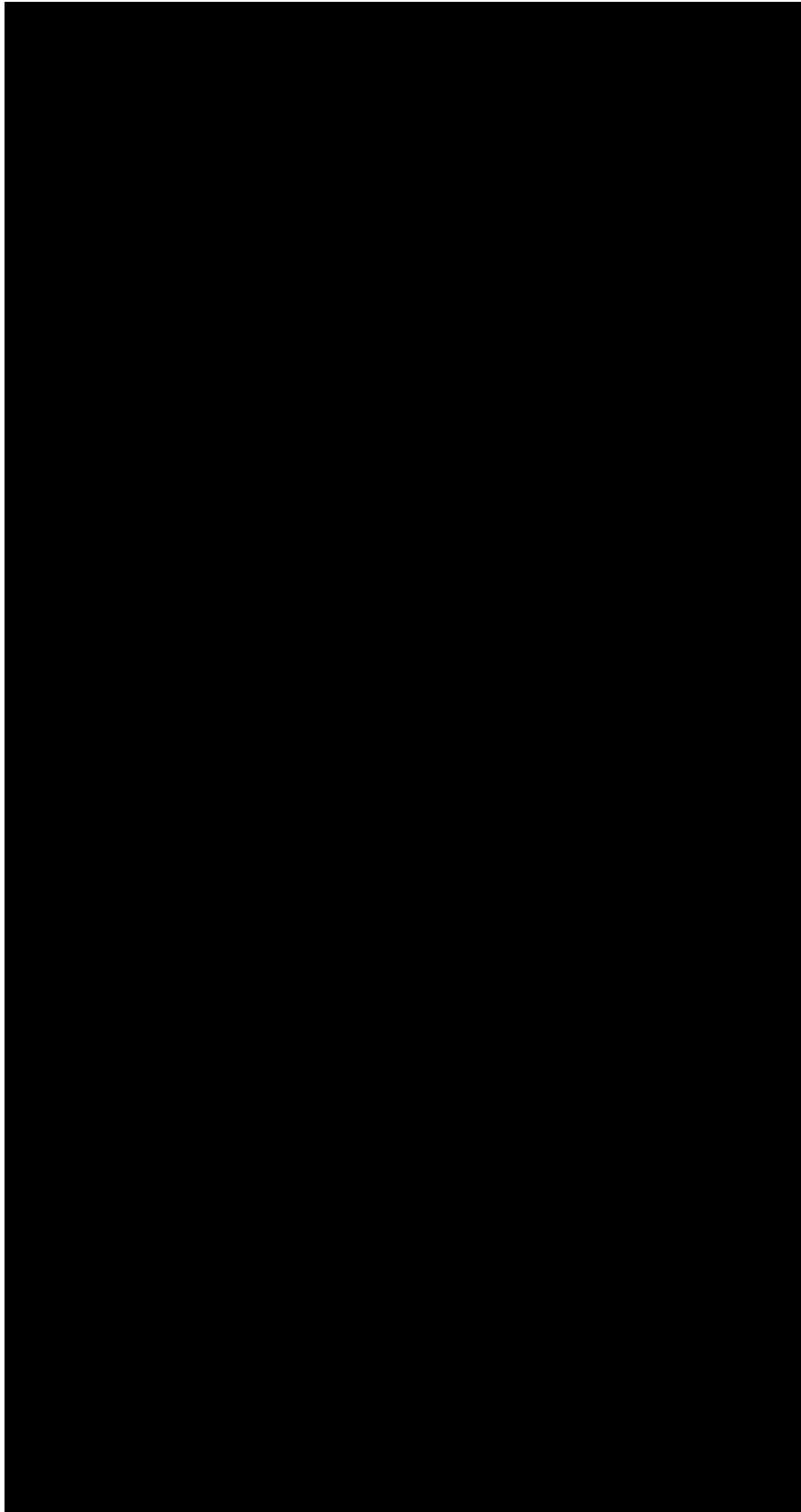
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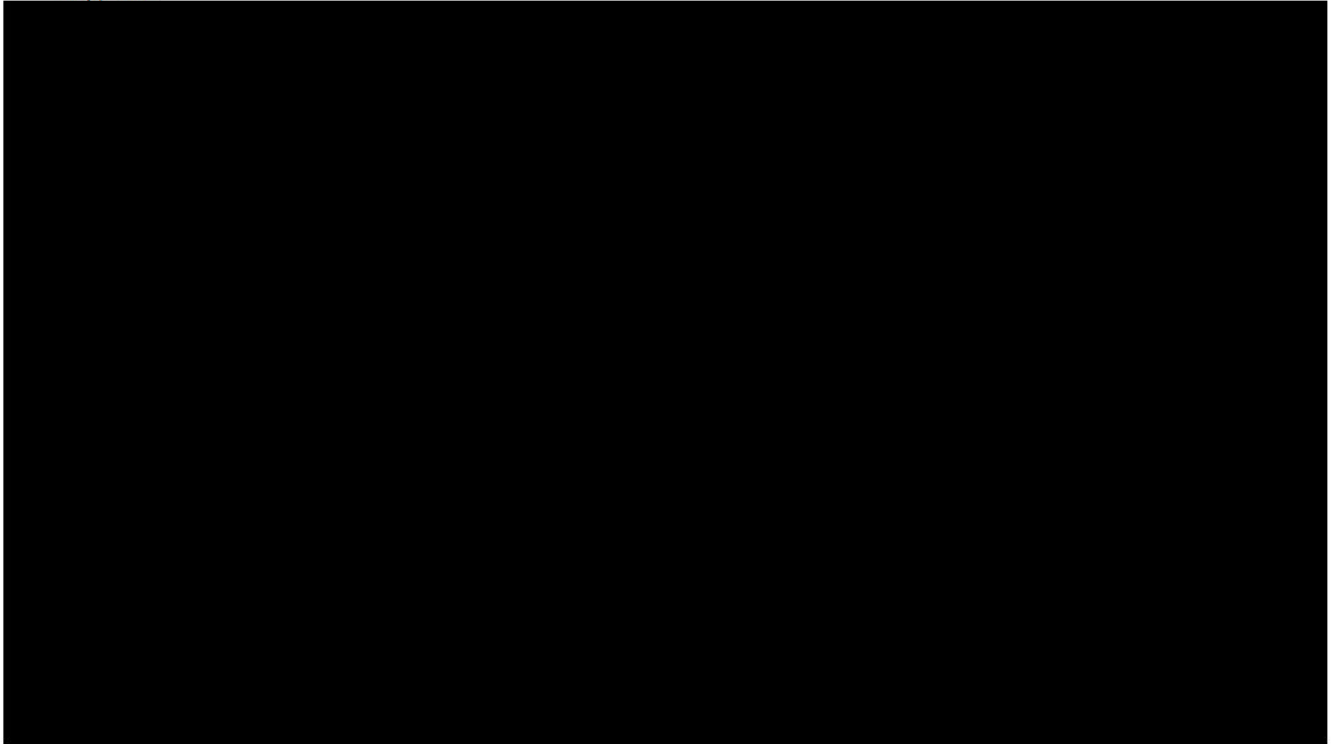


[REDACTED]

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2.3 Morphological Features of New Microorganisms

The modified *S. cerevisiae* strain contains [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] The yeast is used to convert plant starch to ethanol for use in biofuel applications. There are no data that indicate expression of the intergeneric genes, which are metabolic genes, change the morphological features of the modified strain relative to the unmodified strain.

2.4 Physiological Features of New Microorganisms

2.4.1 Ability to [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

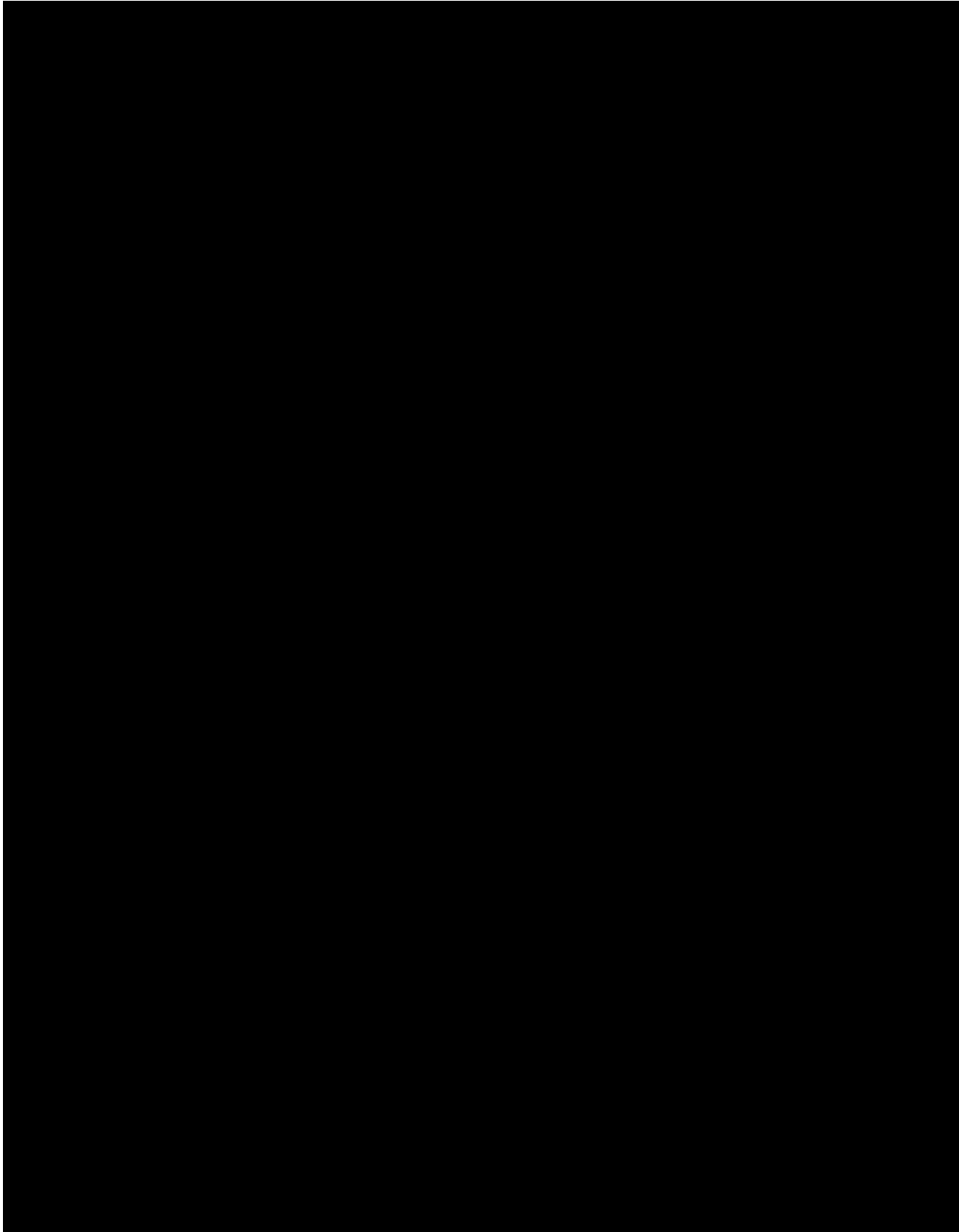
[REDACTED]

[REDACTED]

[REDACTED]

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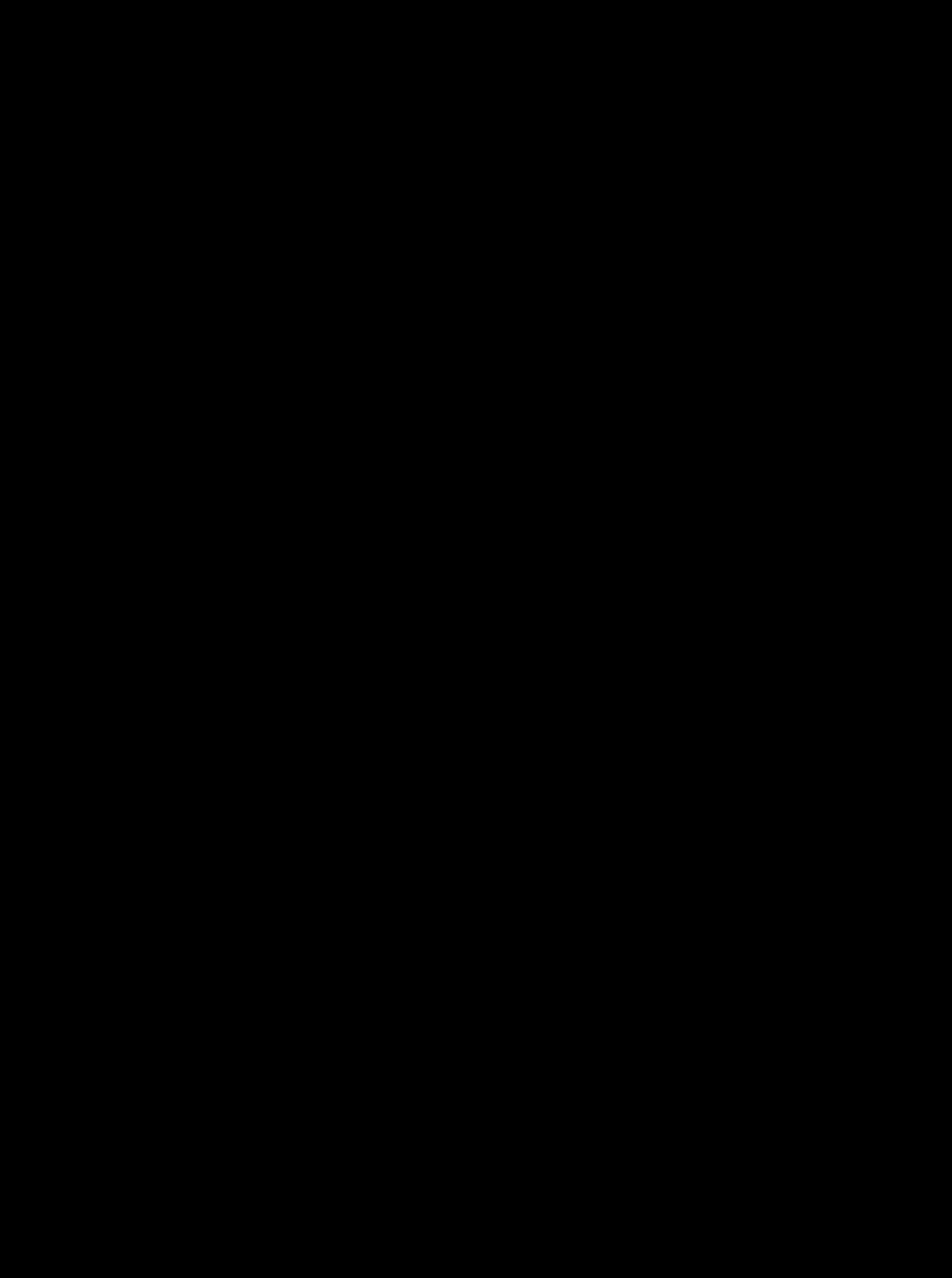
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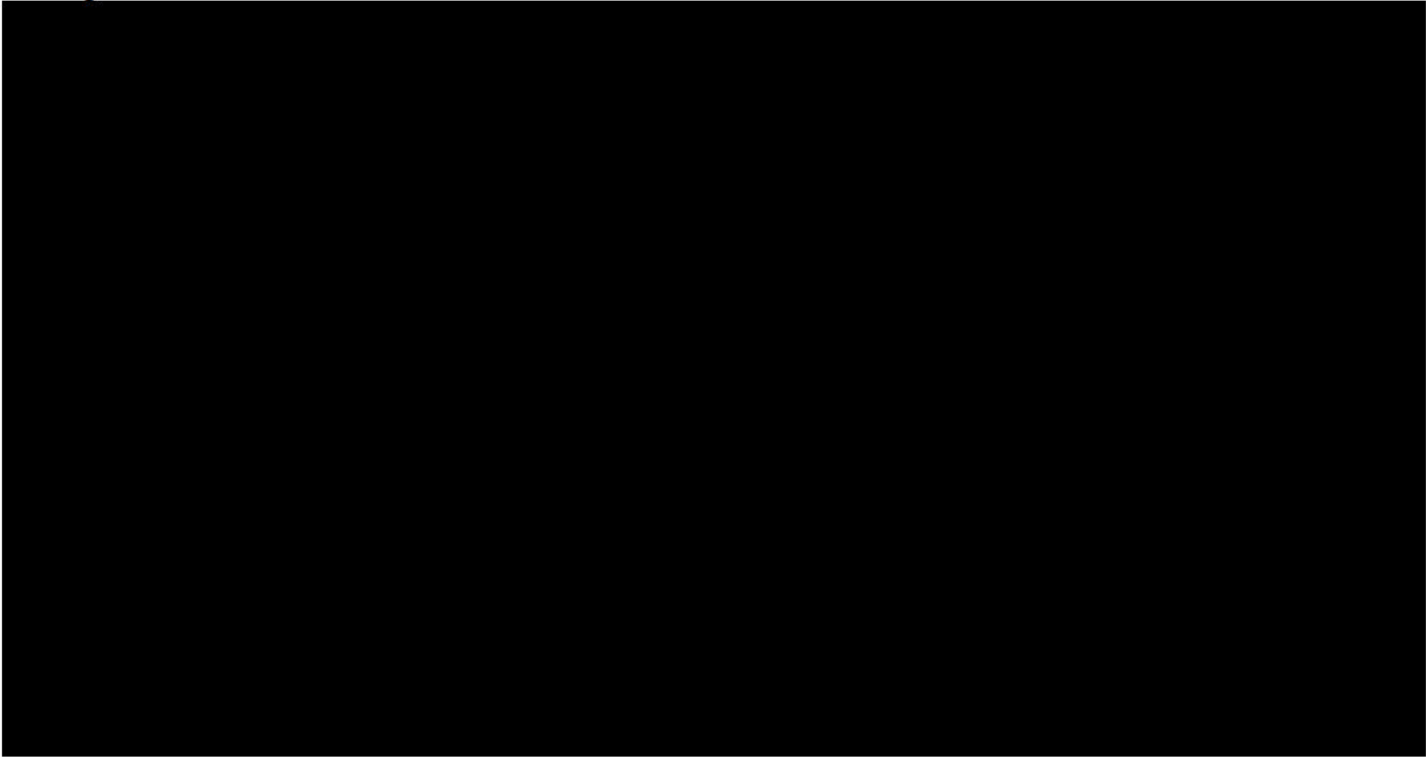


[REDACTED]

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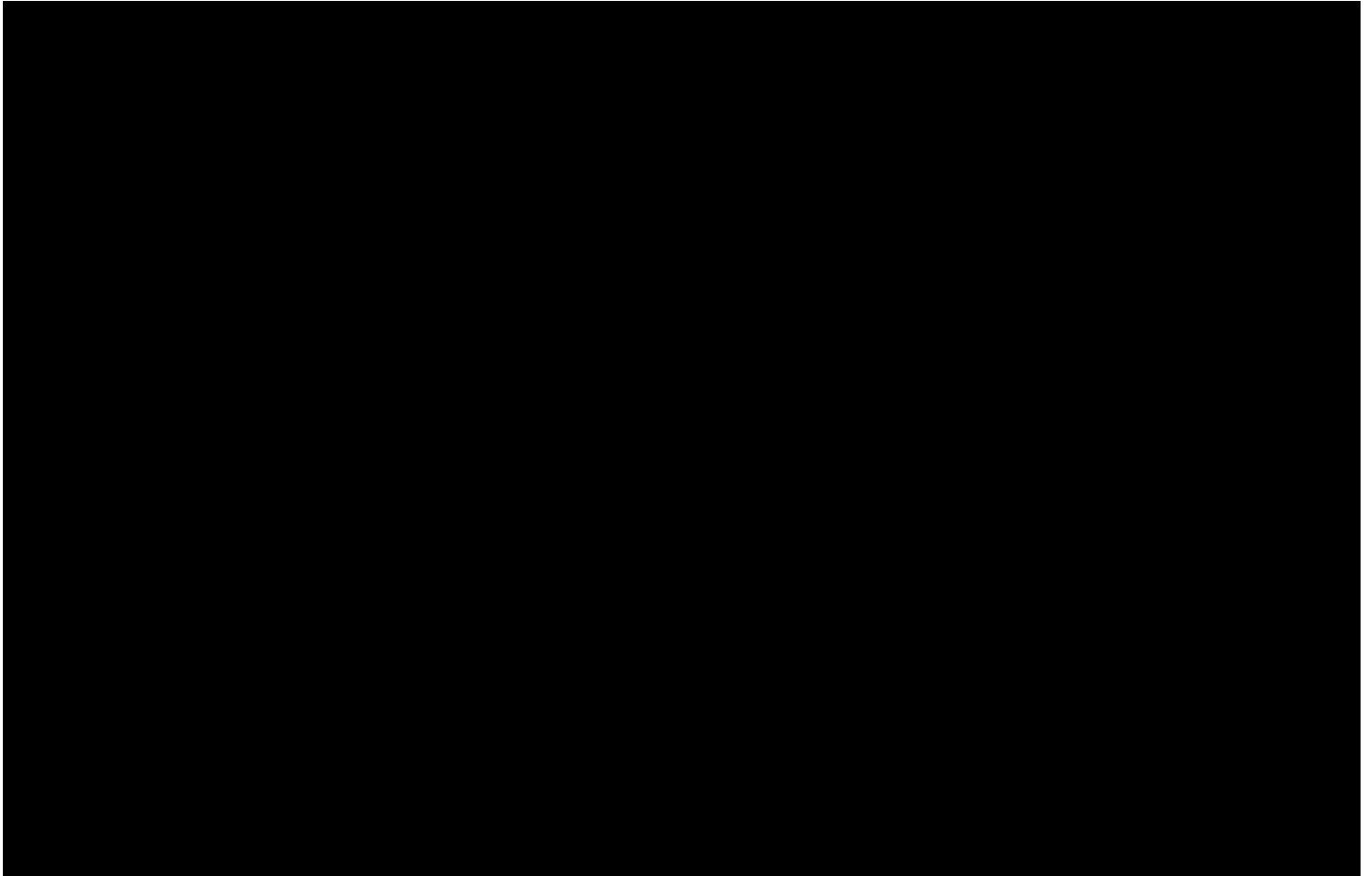
2.4.2 Ability to [REDACTED]



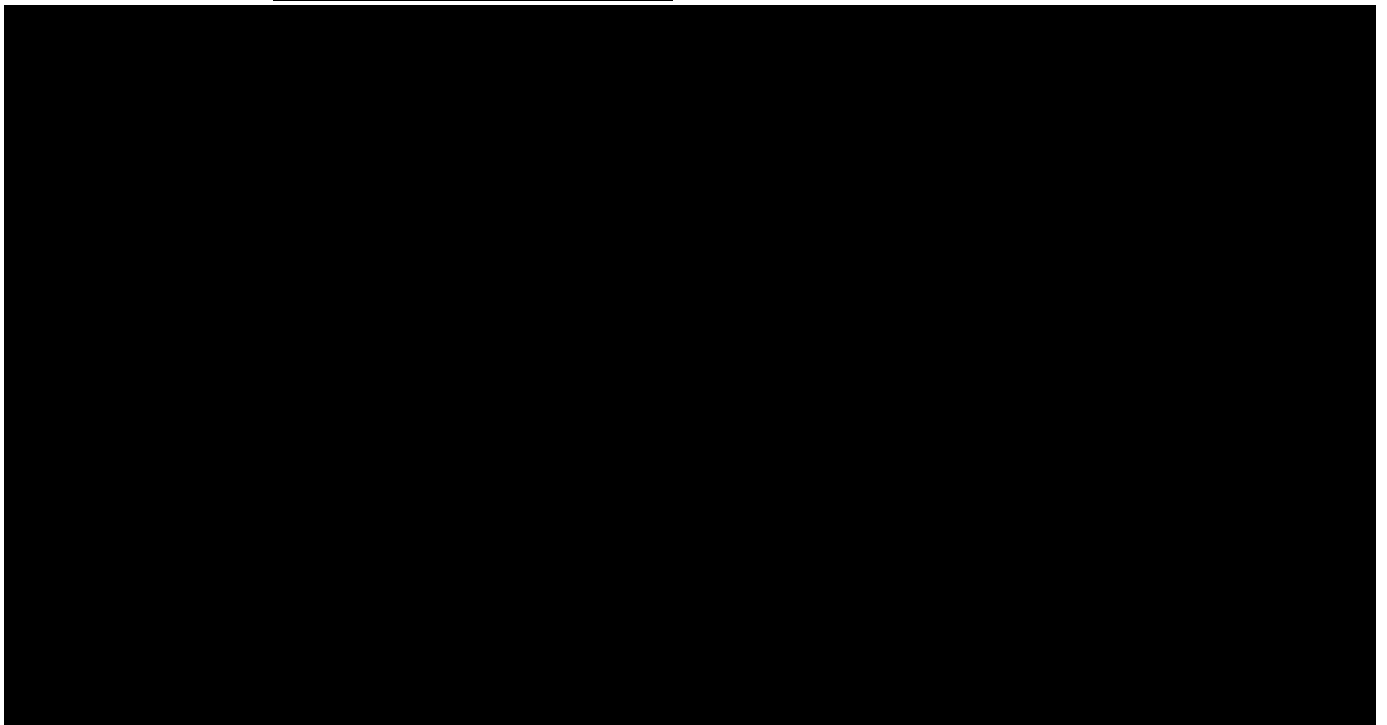
[REDACTED]

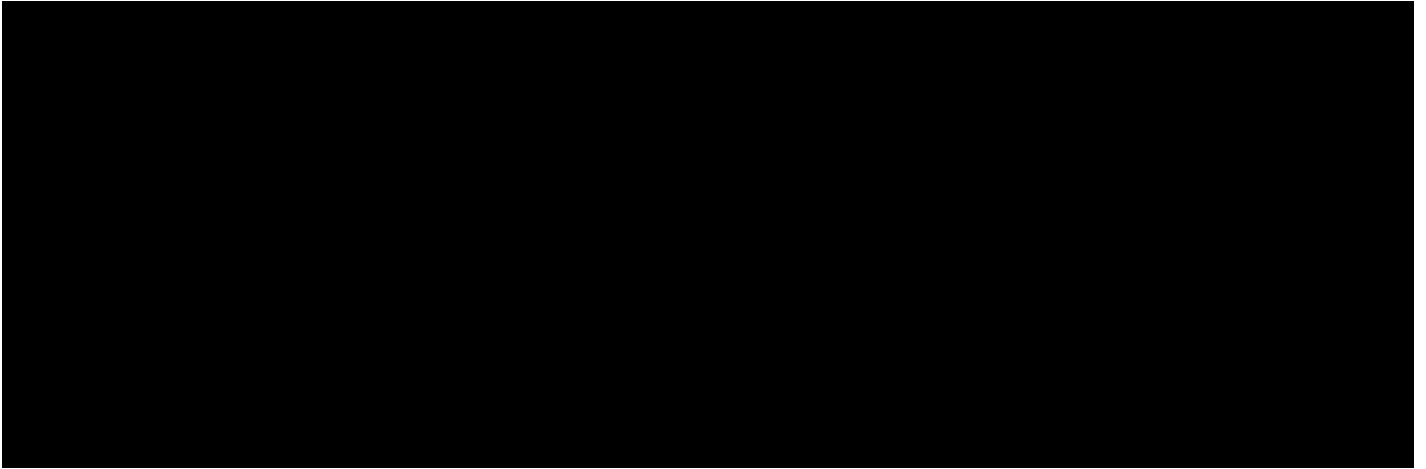
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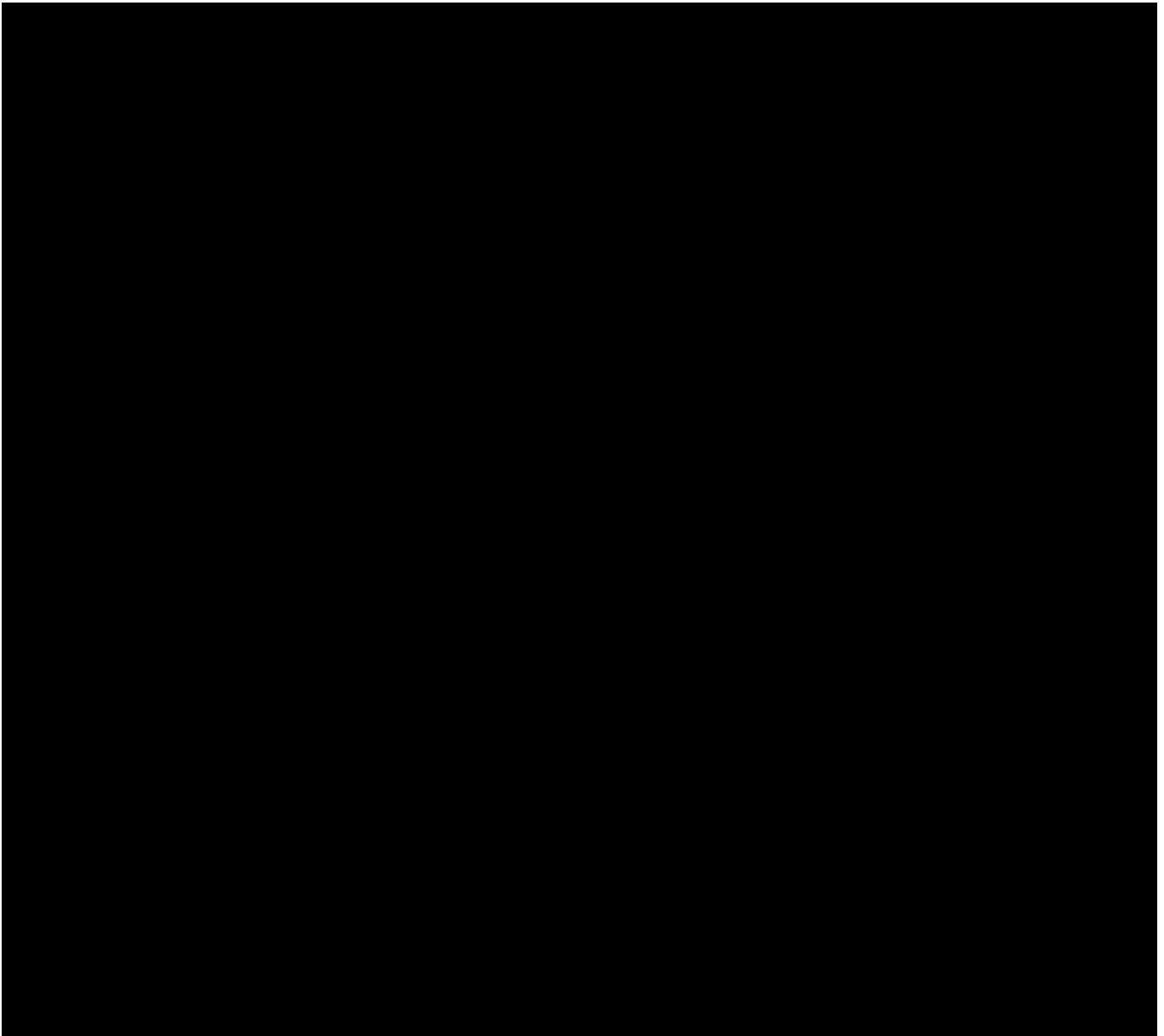


2.4.3 Improved [REDACTED]





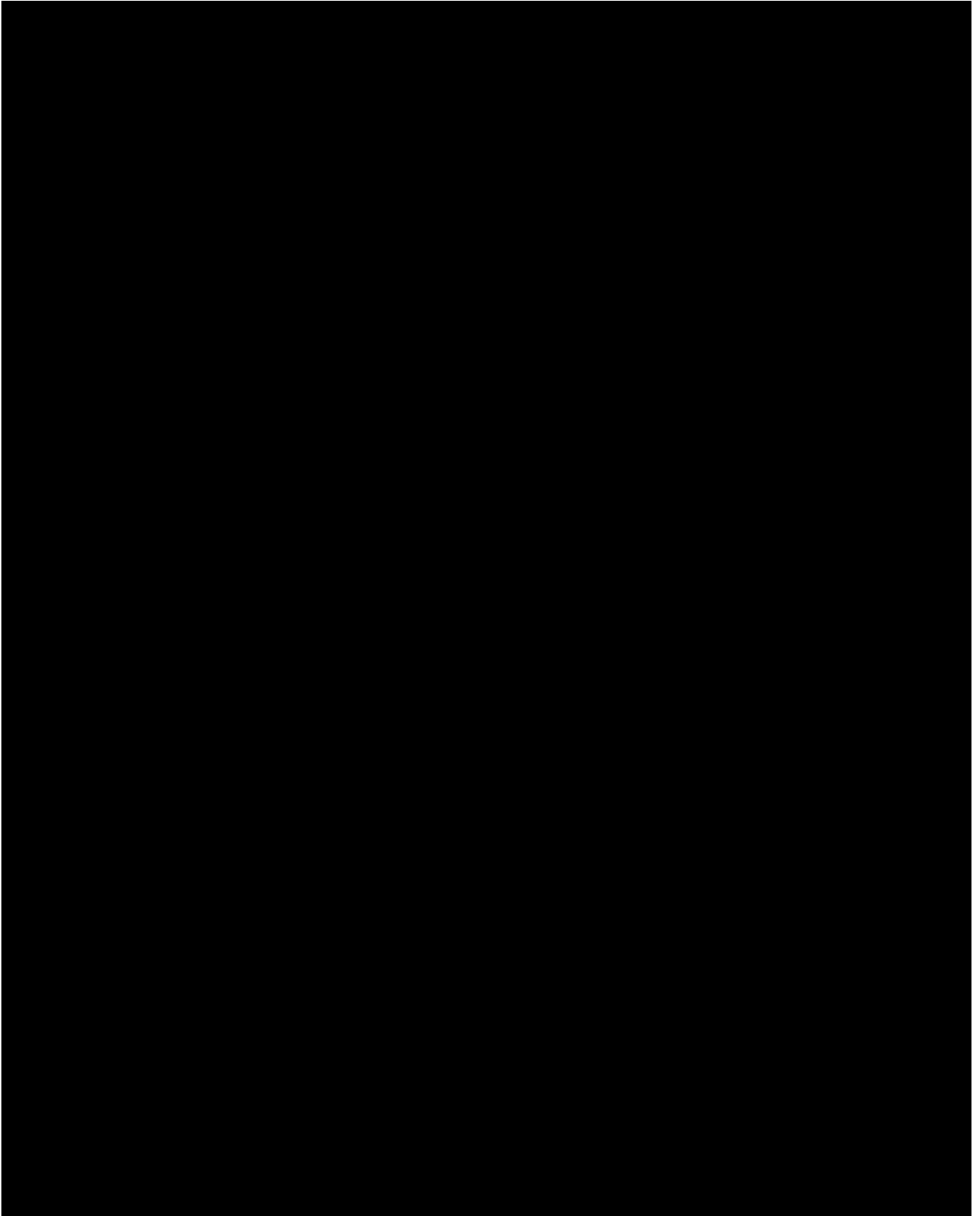
2.4.4 [REDACTED] 1

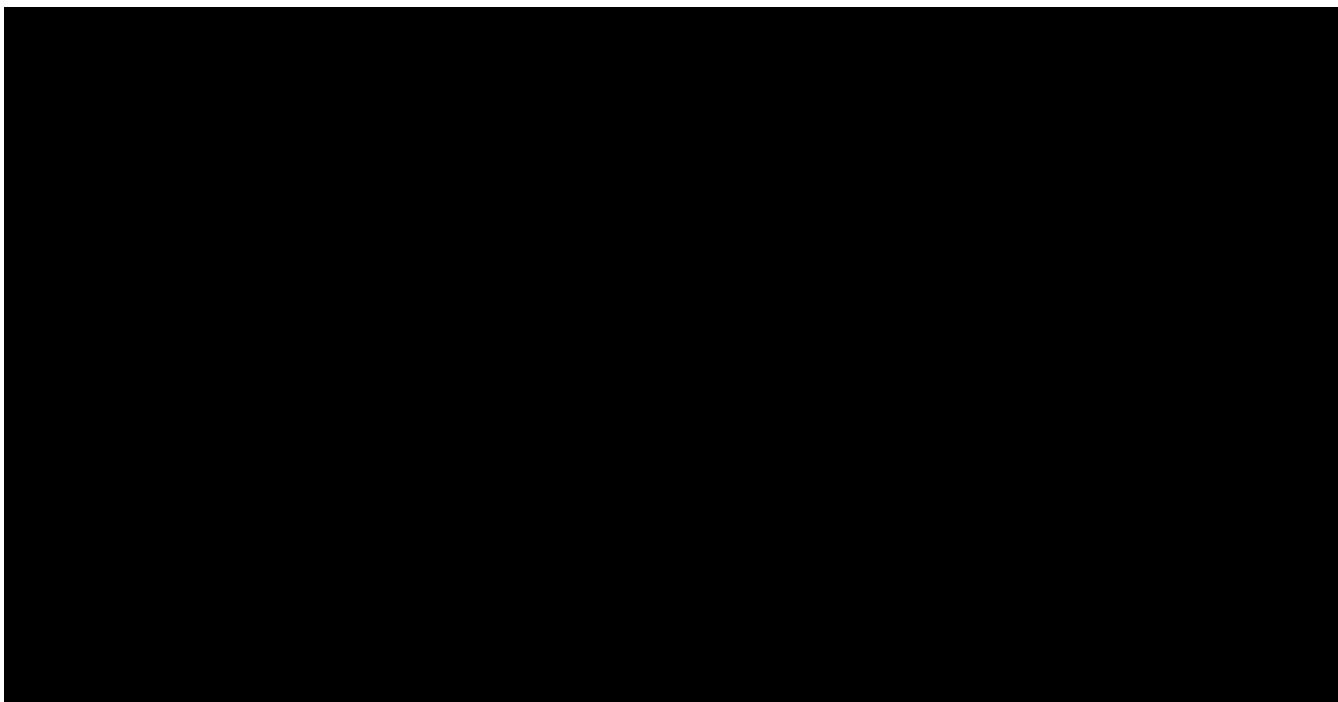


[REDACTED]

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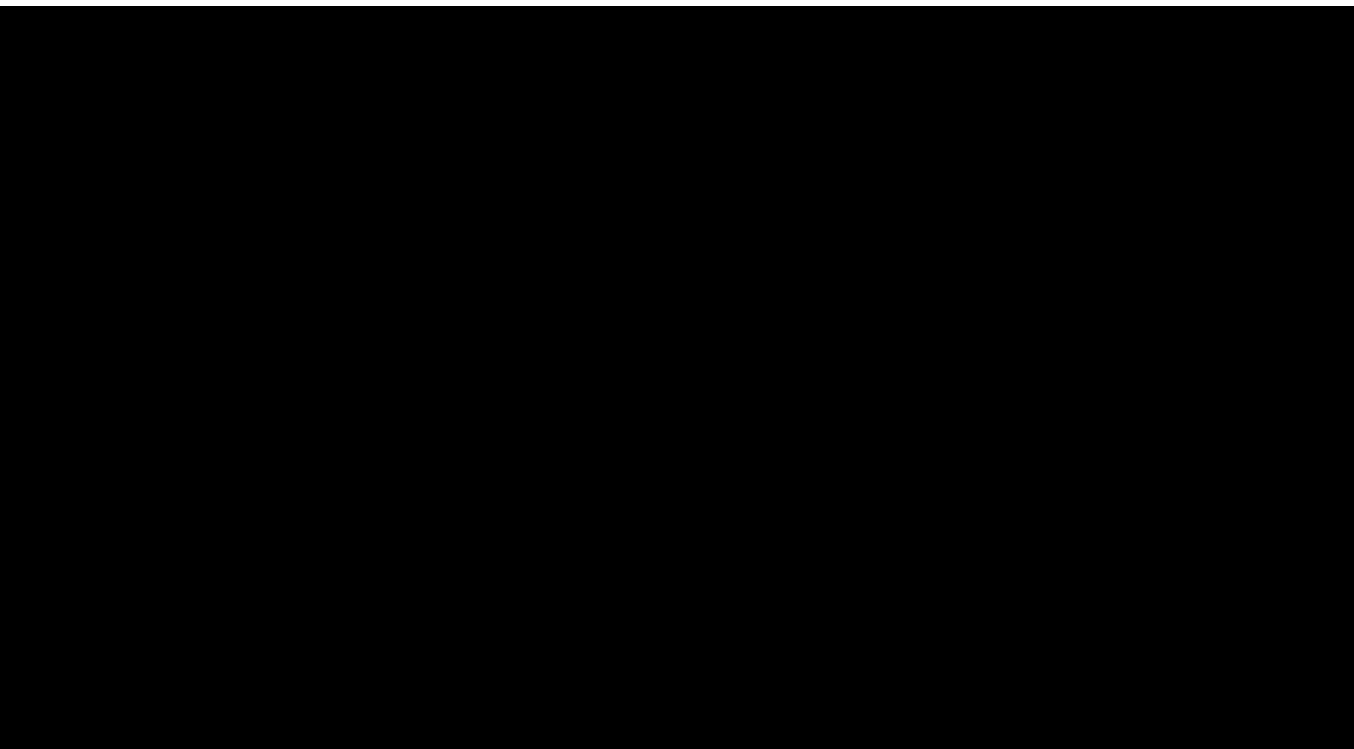
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2.4.5 Fermentation and Growth Characteristics

Laboratory Scale Experiments



2.5 Data by Which the Microorganisms May Be Uniquely Identified and Detected in the Environment

The following approaches can be used to distinguish and detect the modified strain.

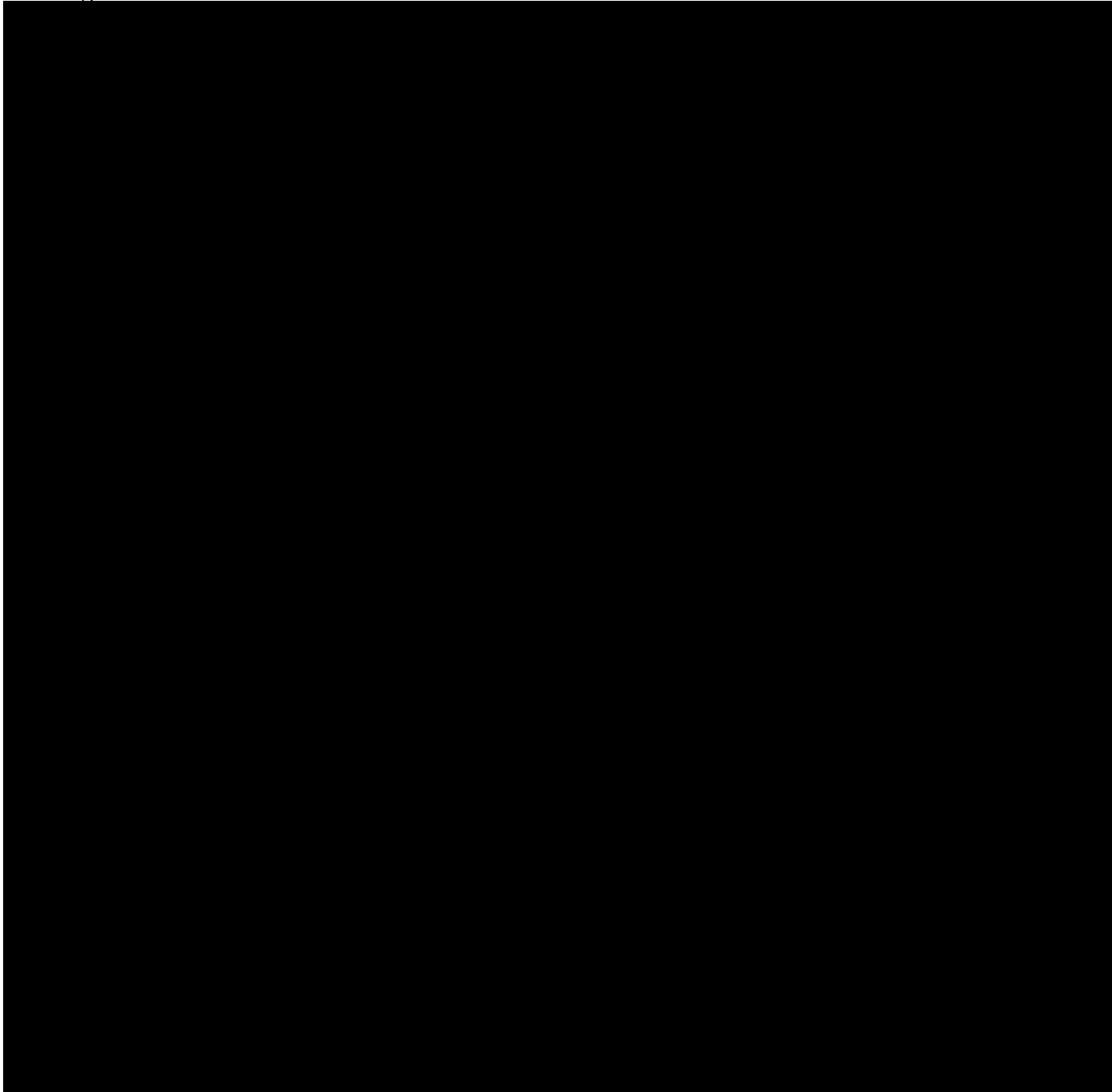
2.5.1 [REDACTED]

[REDACTED]

[REDACTED]

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2.5.3 [REDACTED]

The modified strain can be distinguished from wild type *S. cerevisiae* by [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

2.5.4 PCR

[REDACTED]
[REDACTED]
[REDACTED]

2.6 Description of Traits for Which the Microorganisms Were Selected

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

2.7 Detailed Description of the Genetic Construction

Genetic Construction of [REDACTED]

The modified strain [REDACTED] was constructed as outlined in **Figure 1** in **Section 2.2**. The molecular tools and practices used during the construction of the MCAN strain are standard to the field of biotechnology and yeast genetics. Information regarding the engineering of the strain in **Figure 1** are described in the sections below.

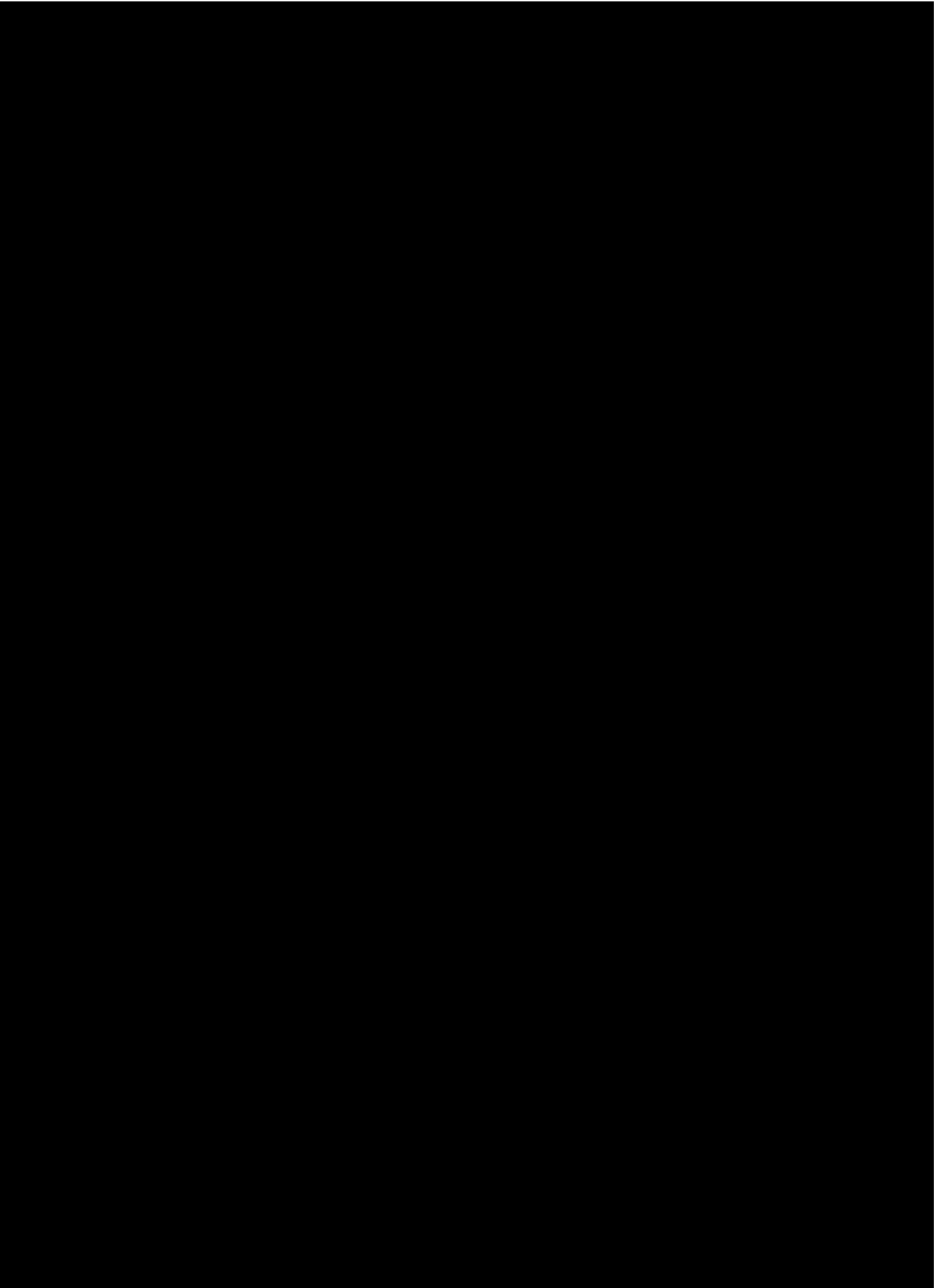
2.7.1 Genetic Construction Details [REDACTED]

[REDACTED]

[REDACTED]

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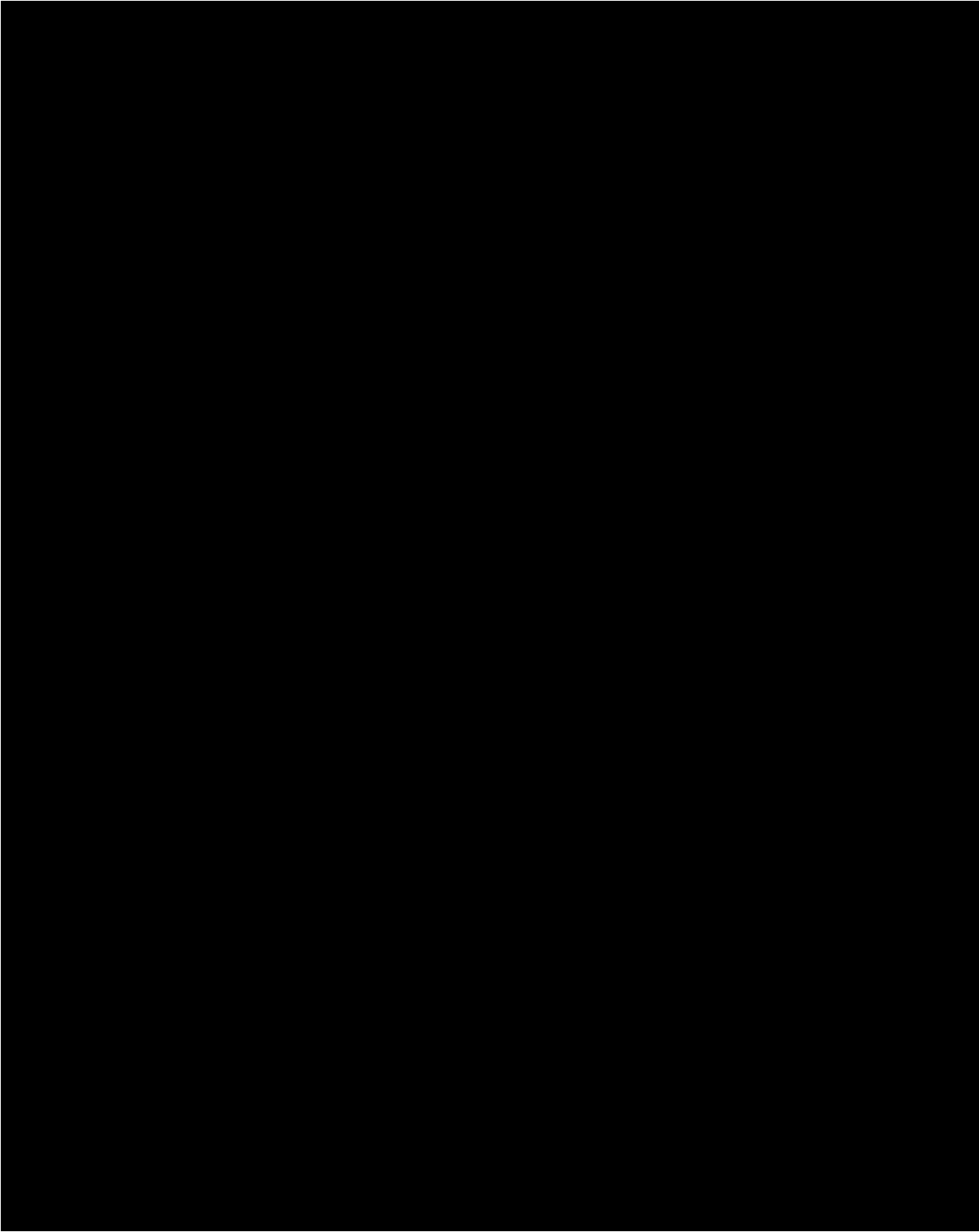
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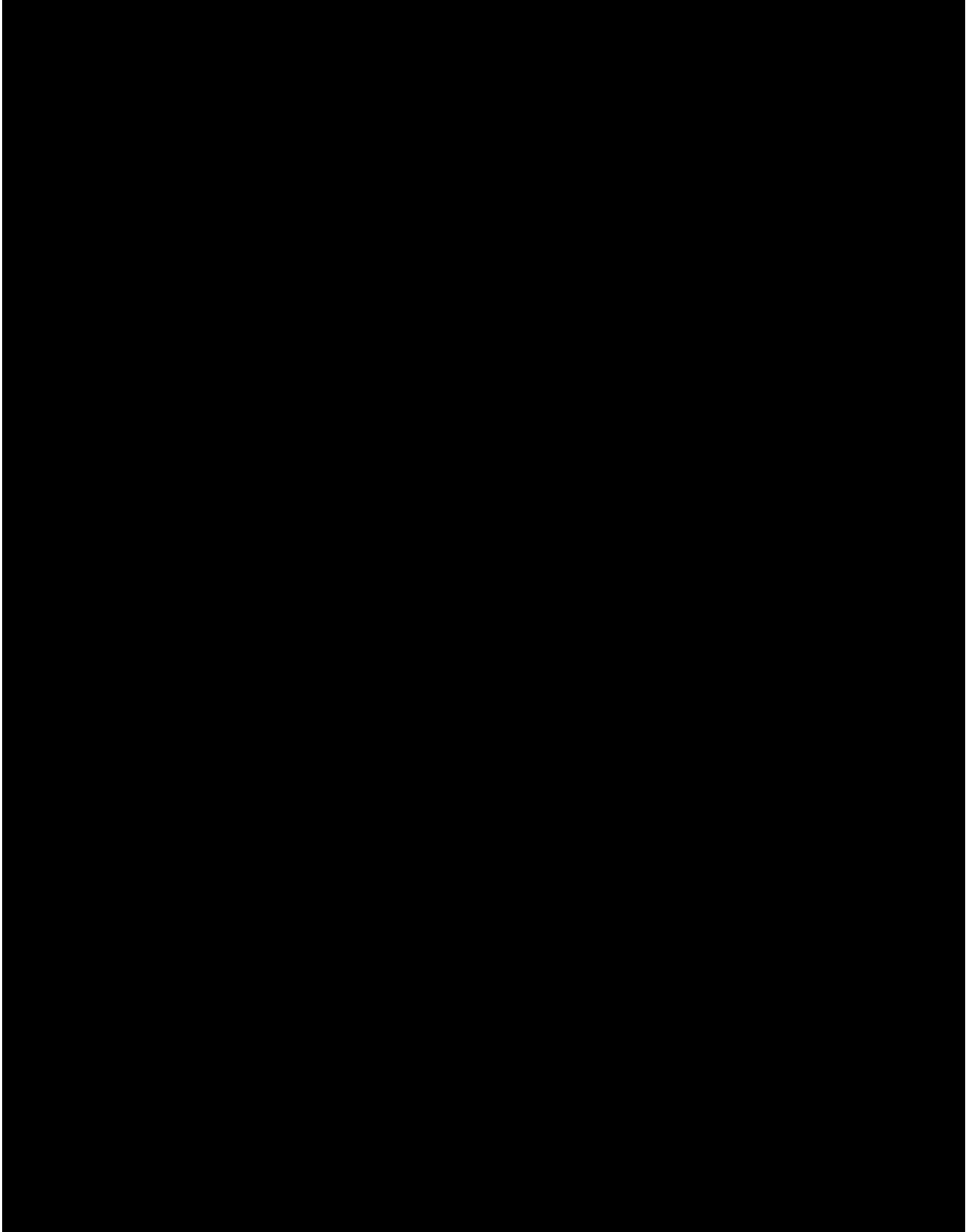


[REDACTED]

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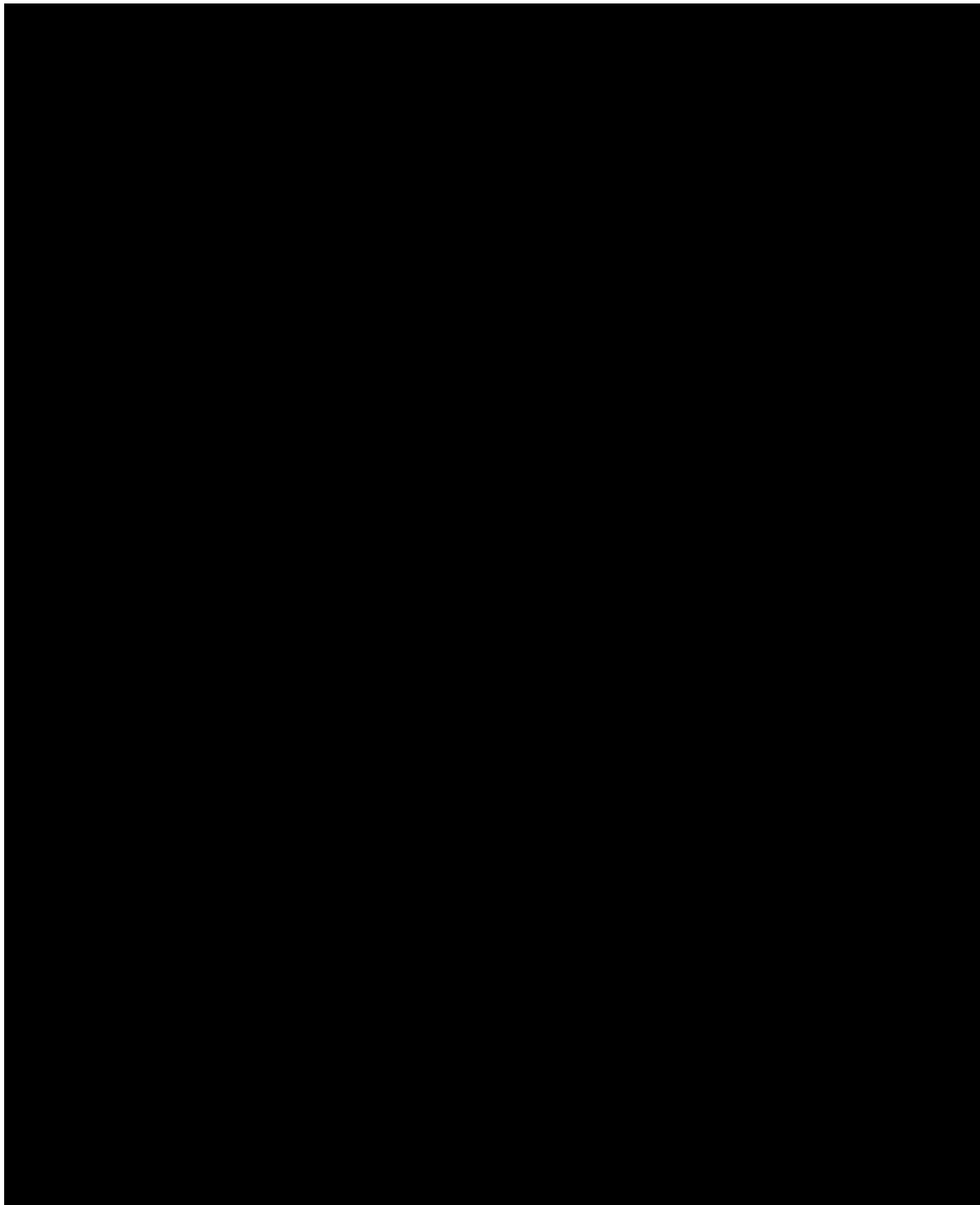




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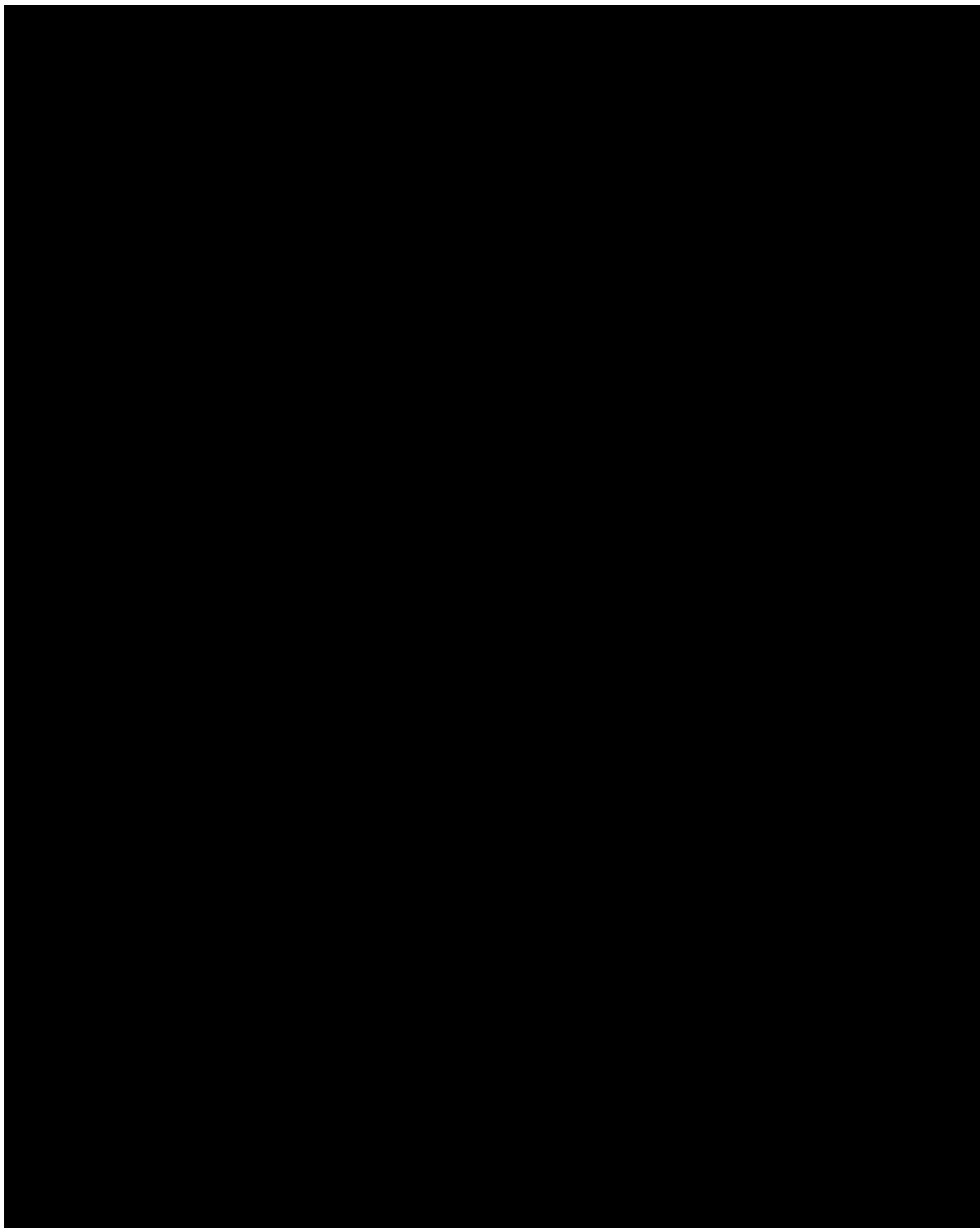
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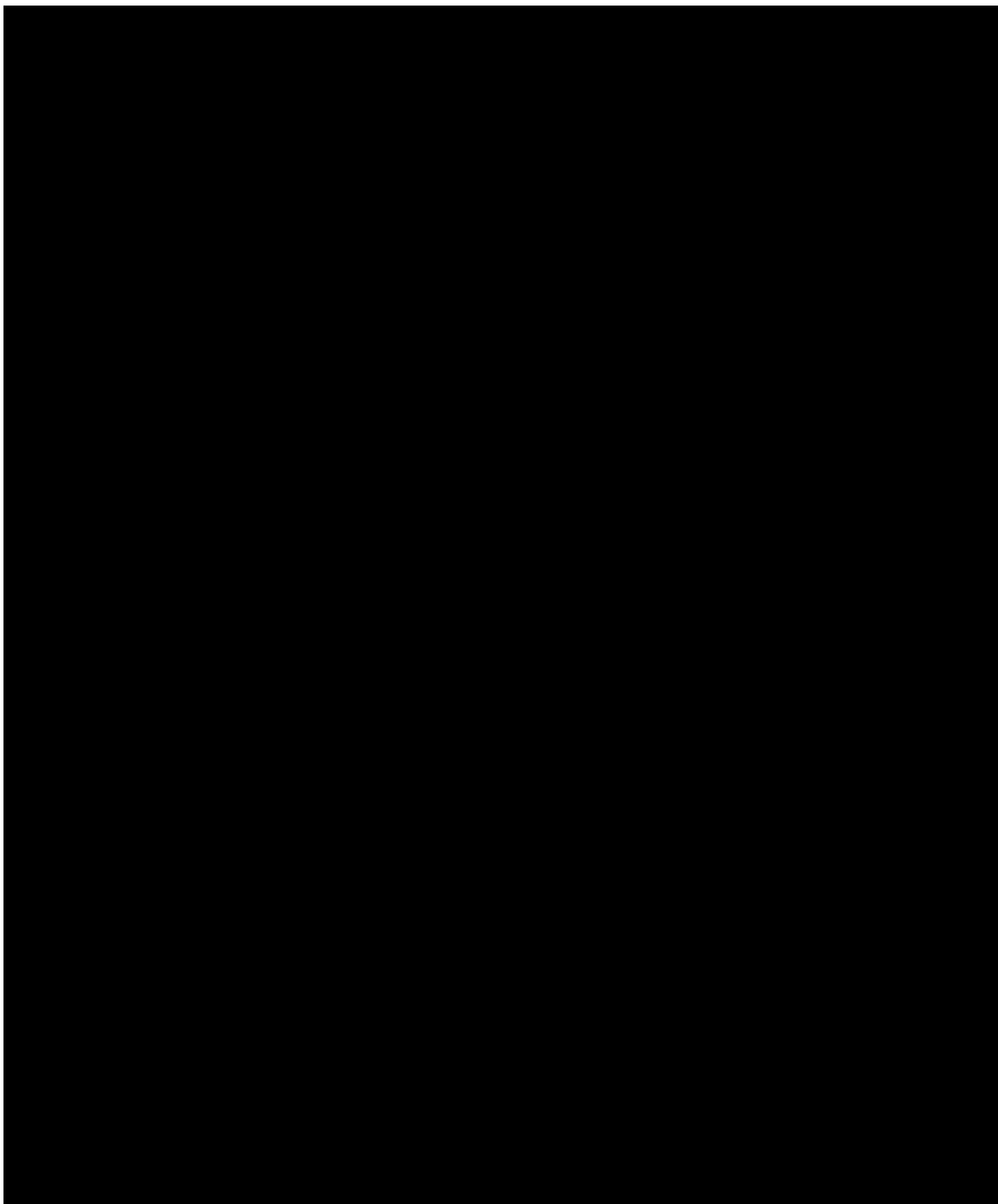
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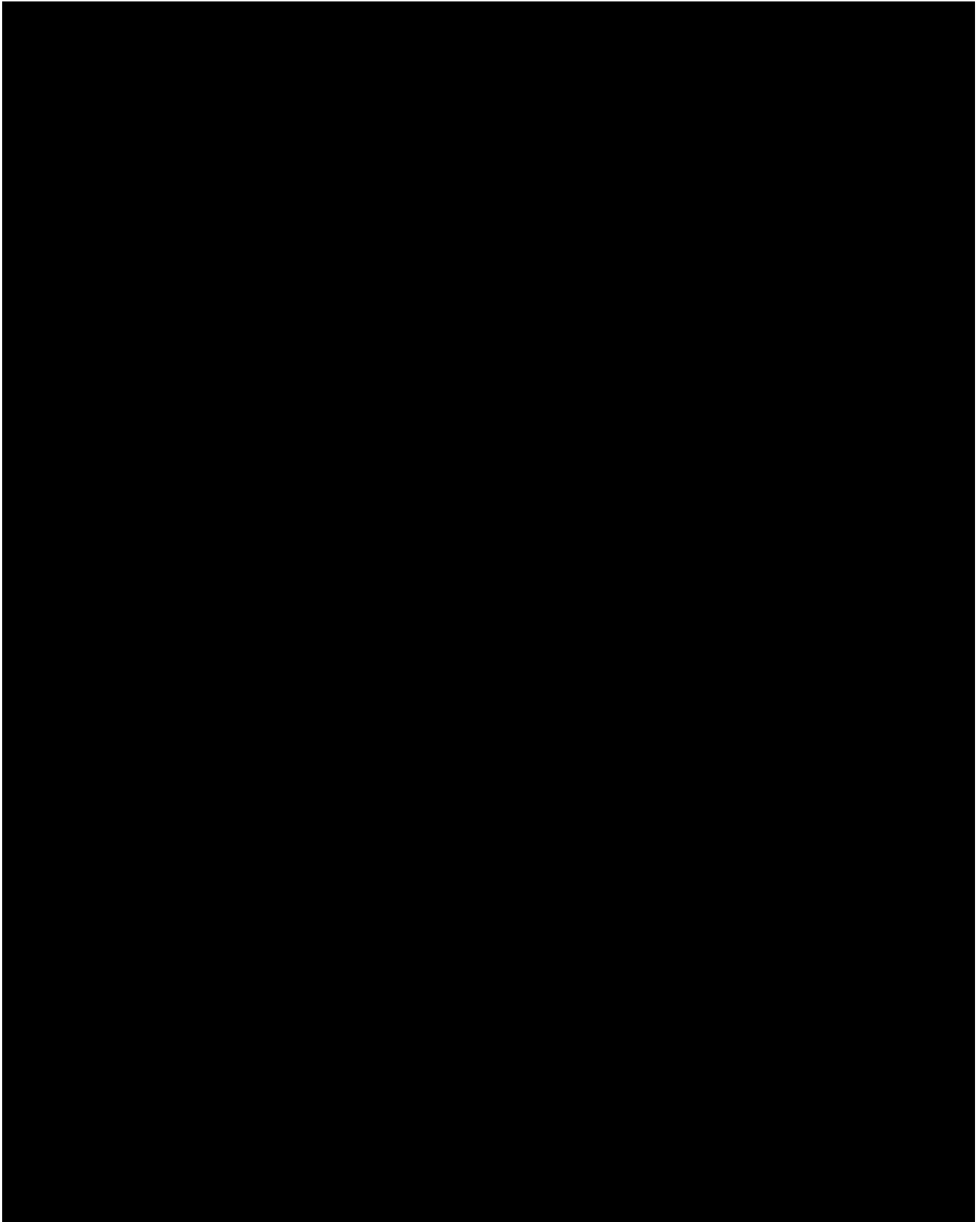
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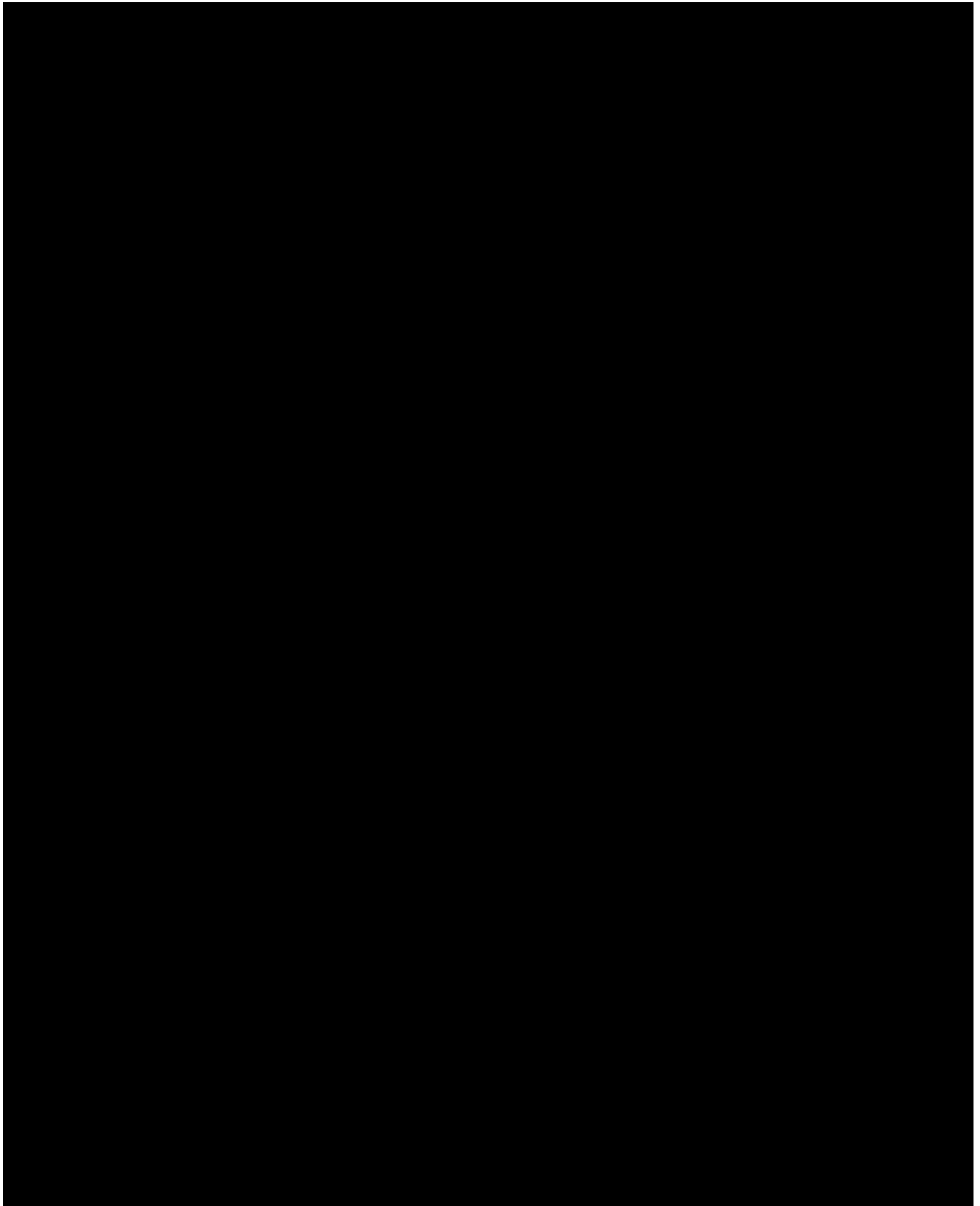
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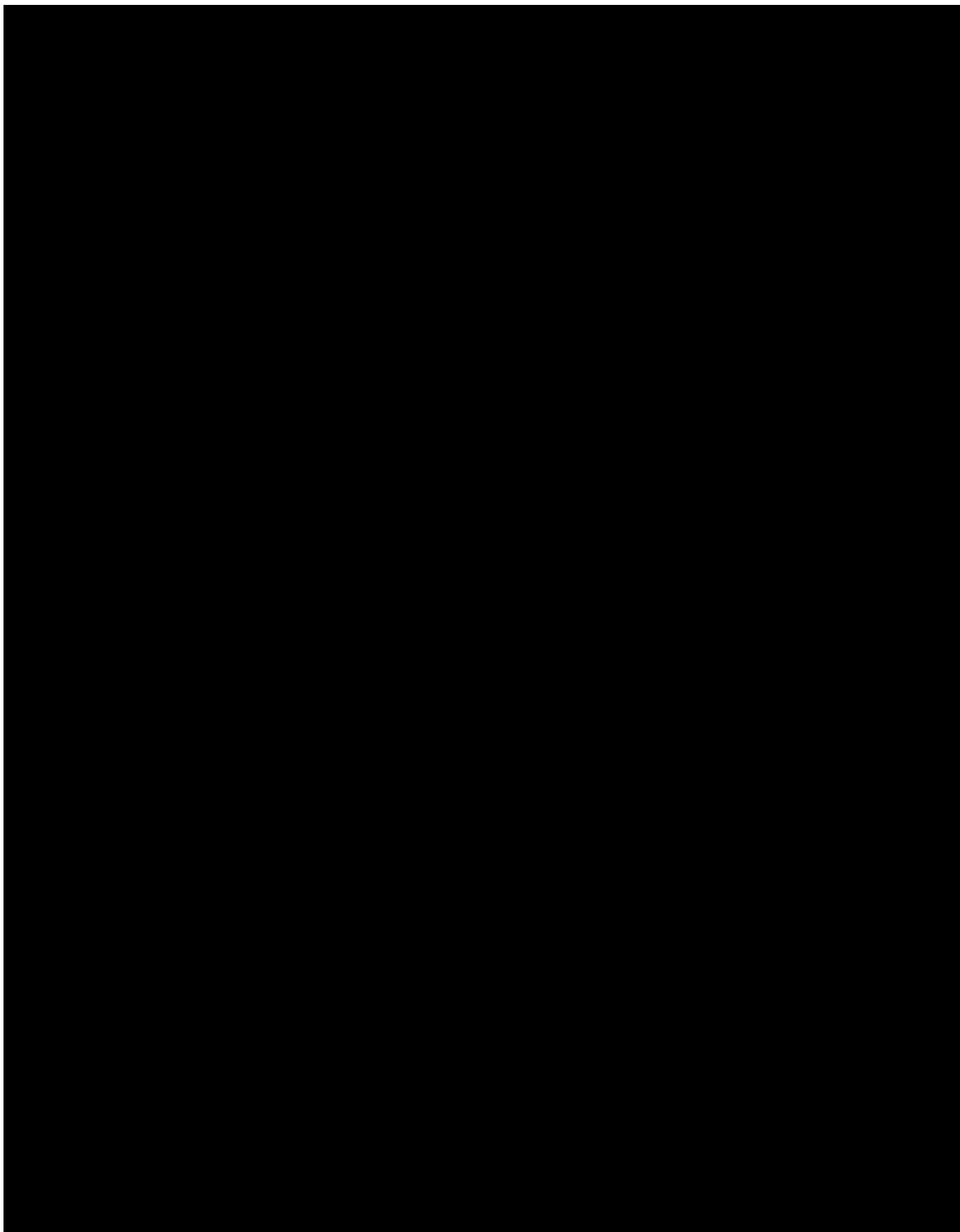
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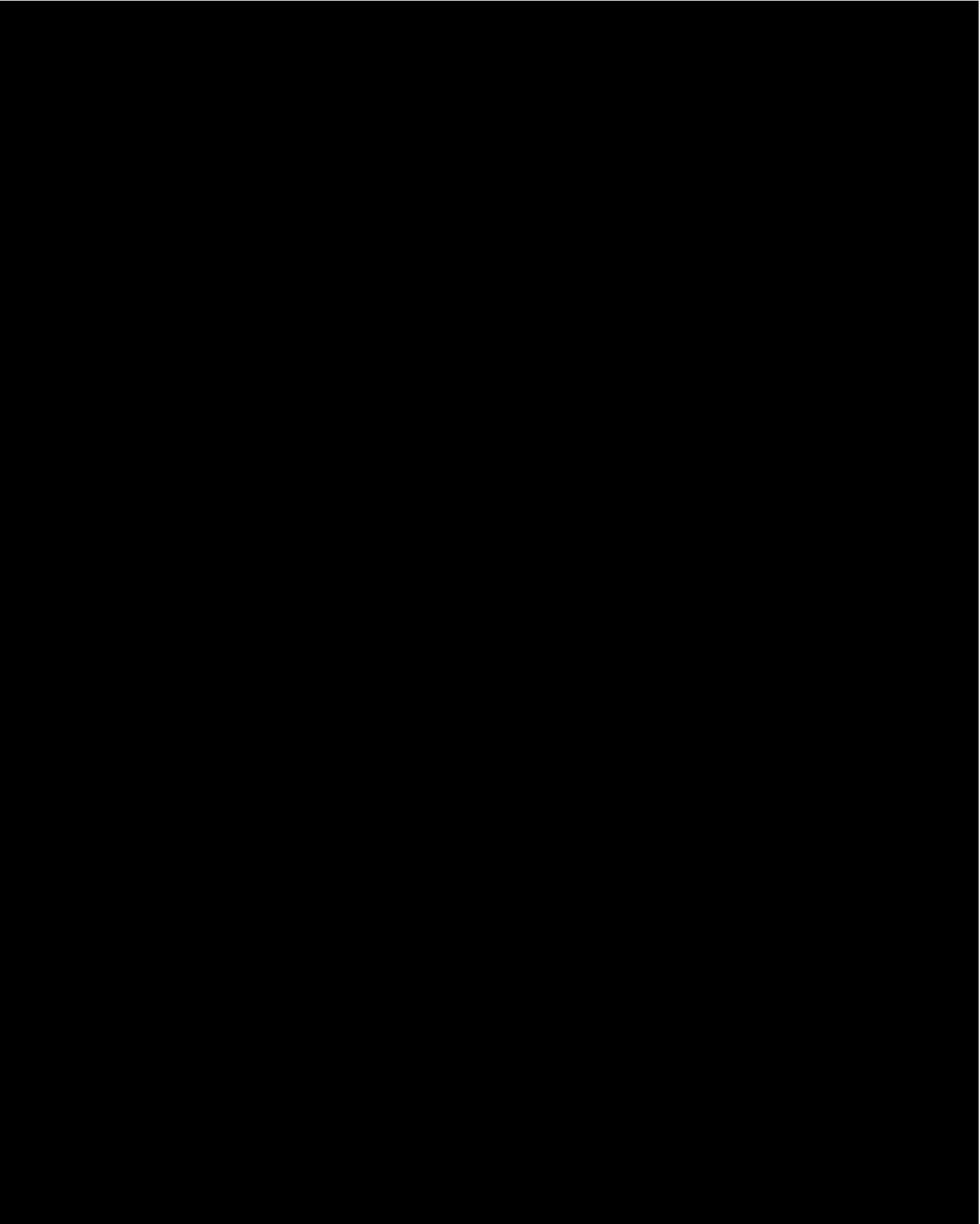
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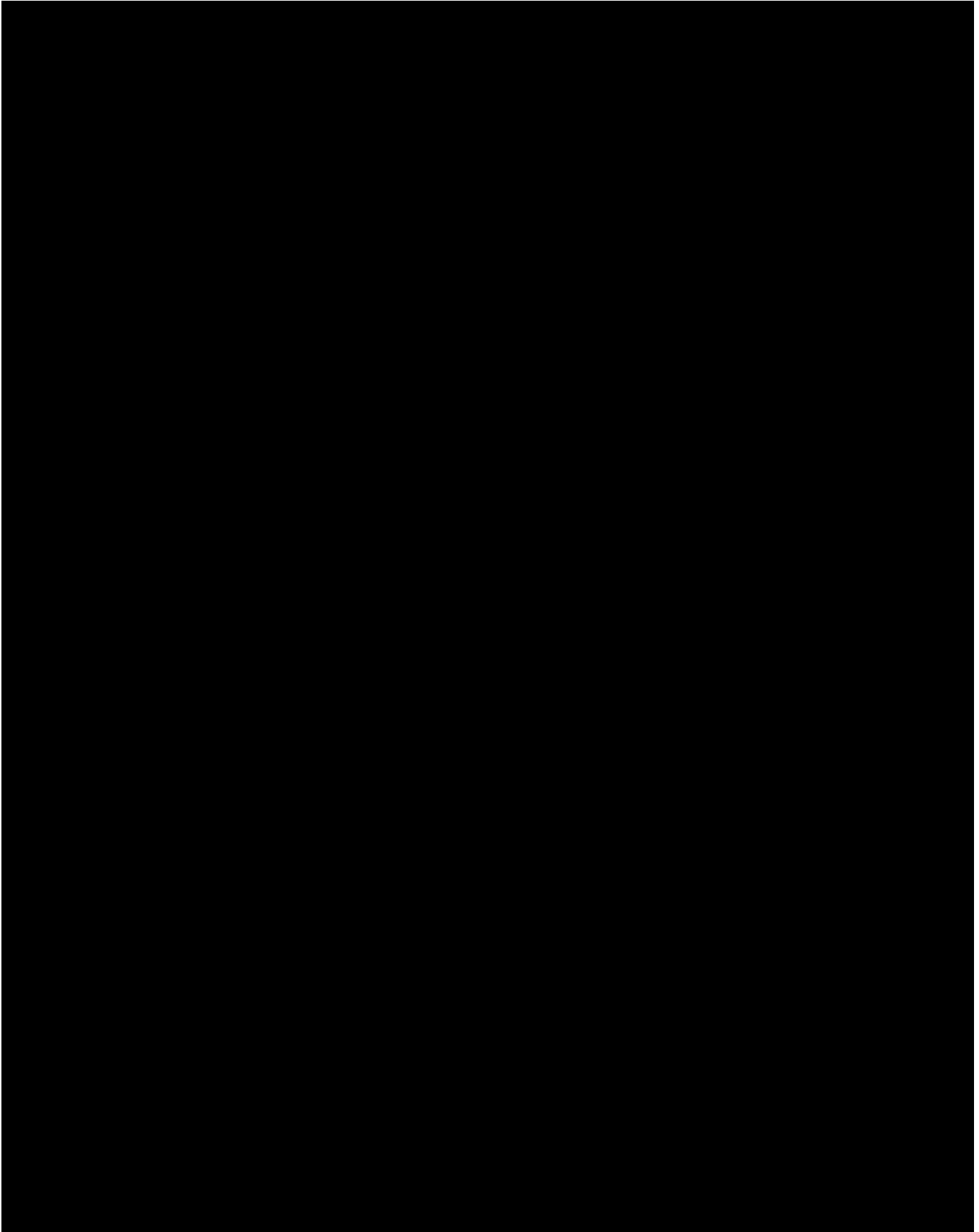
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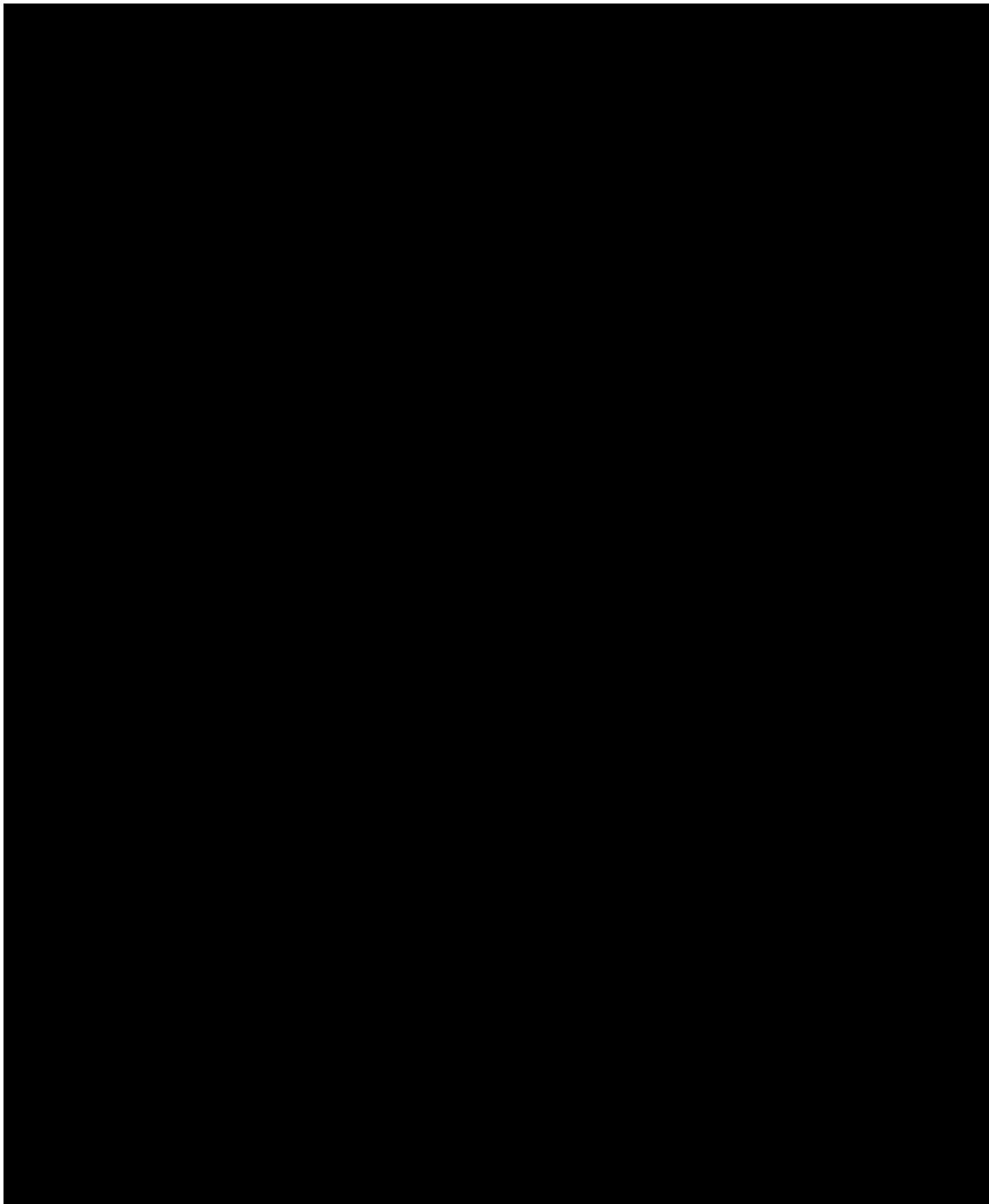
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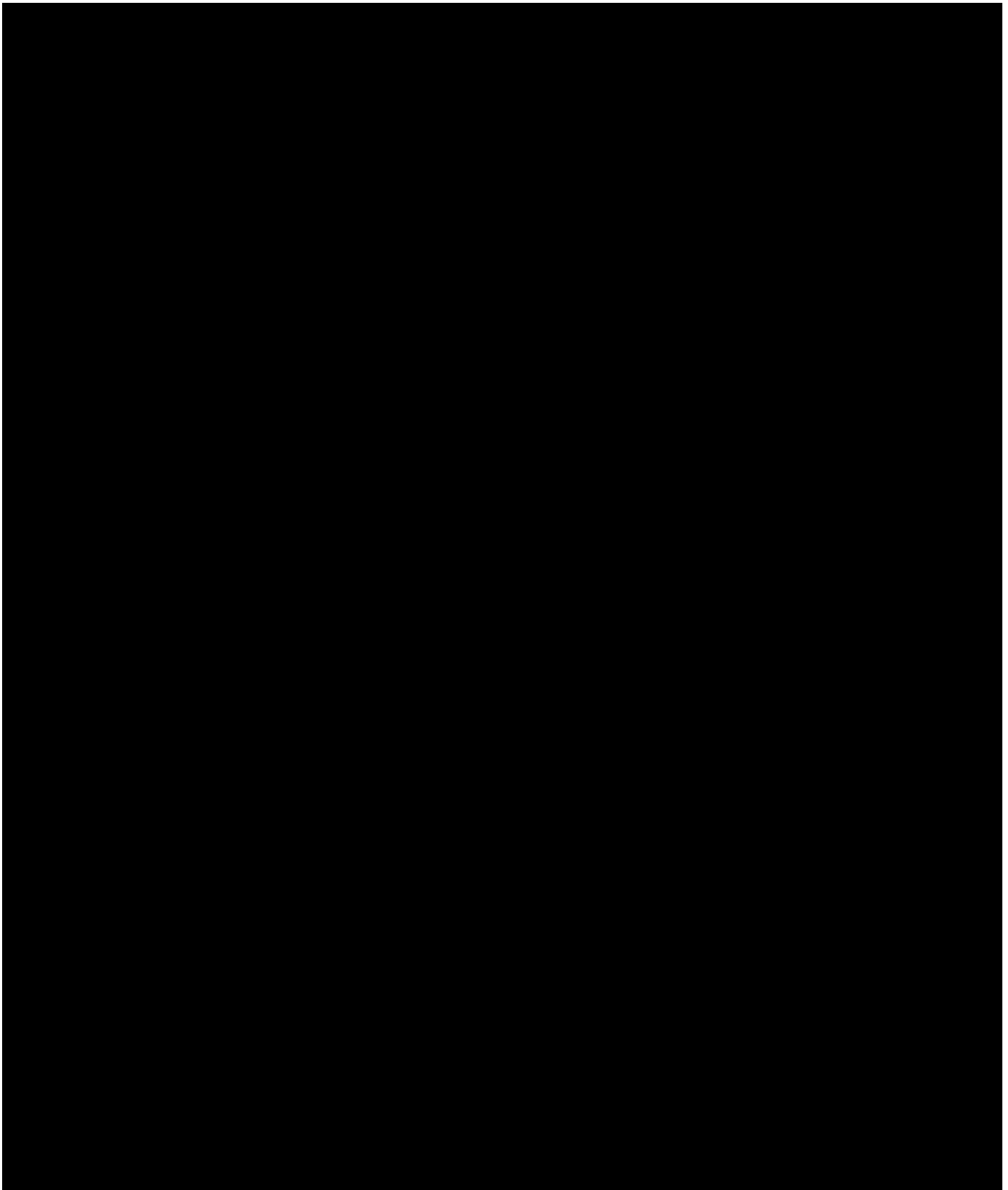
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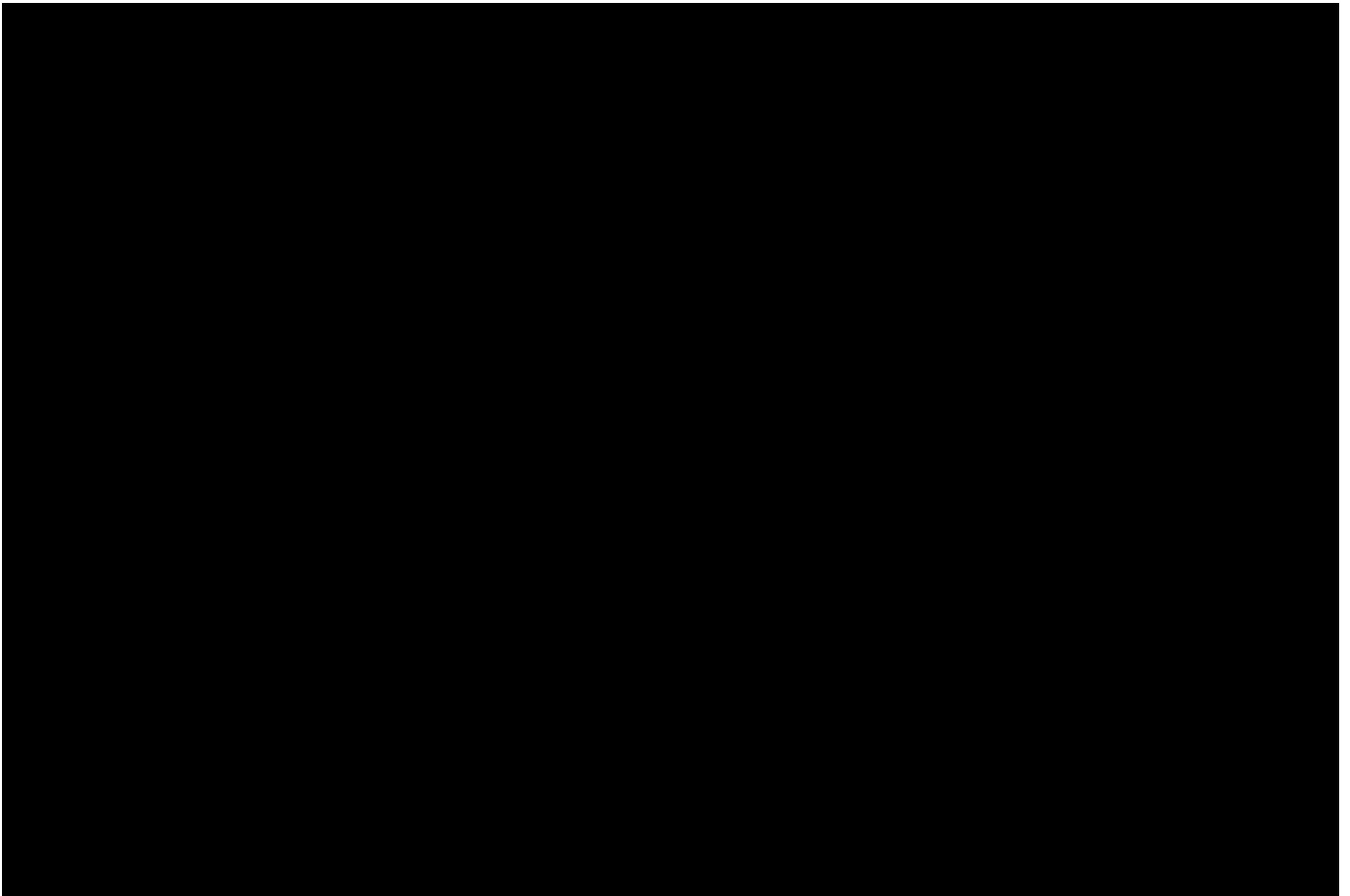
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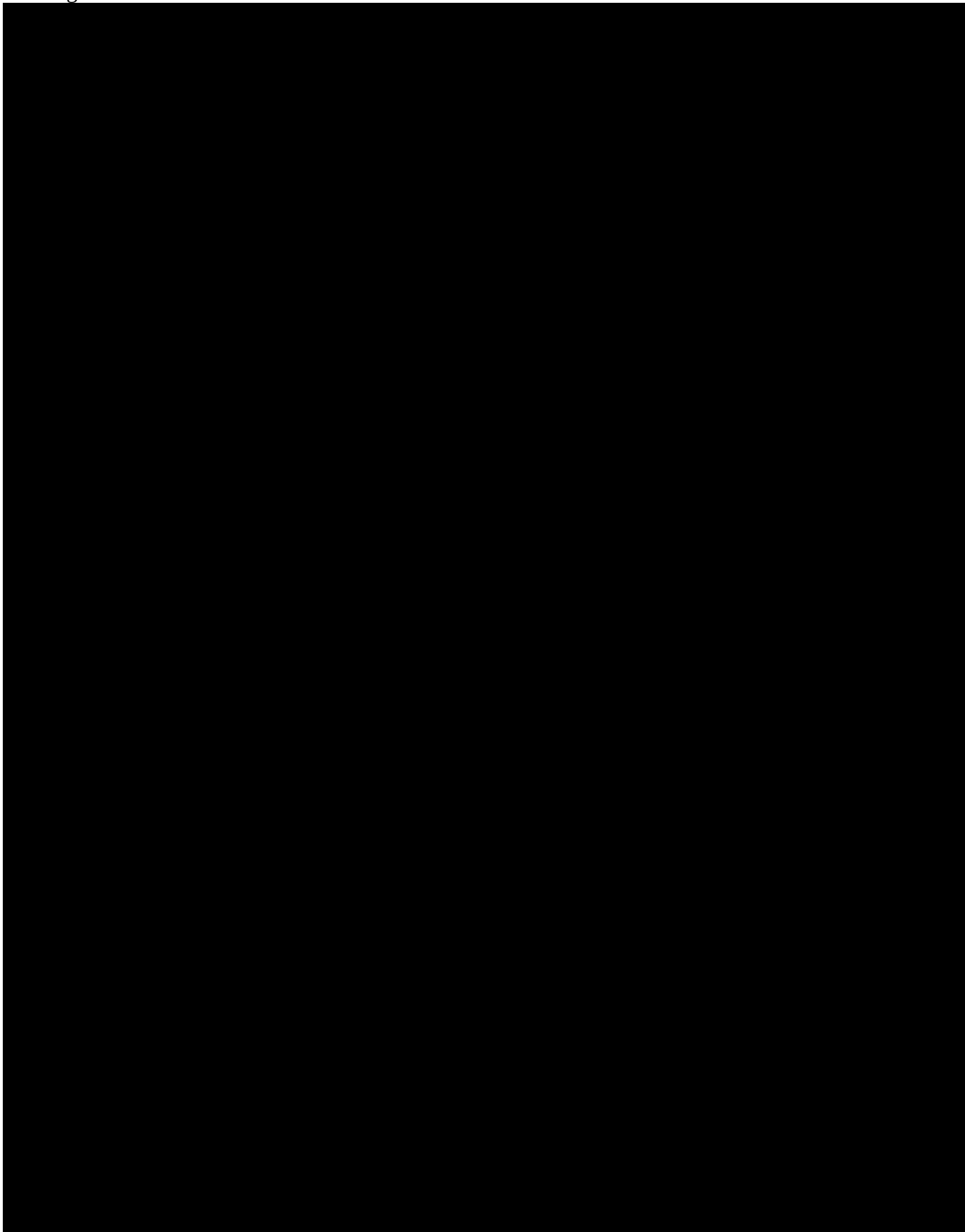
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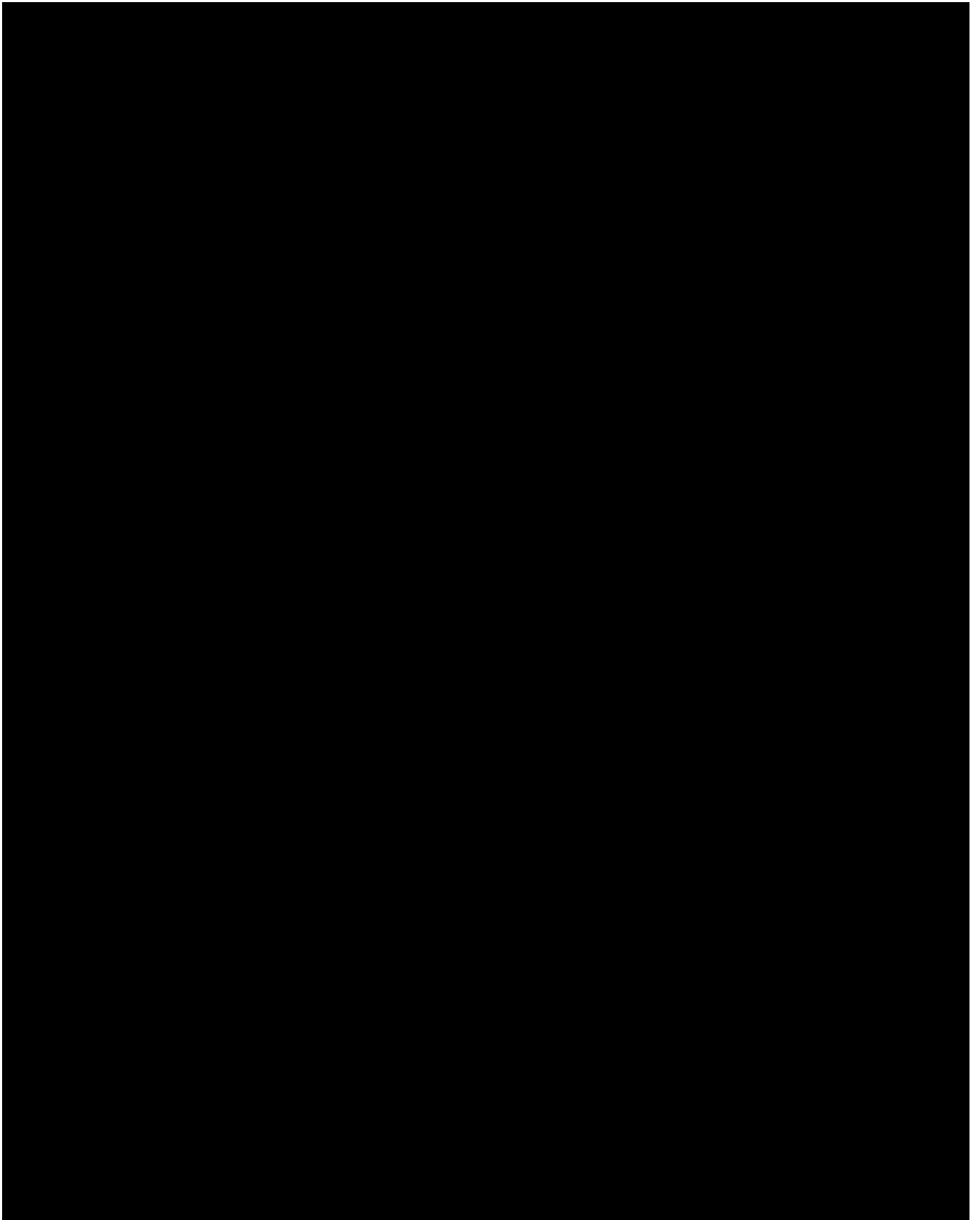
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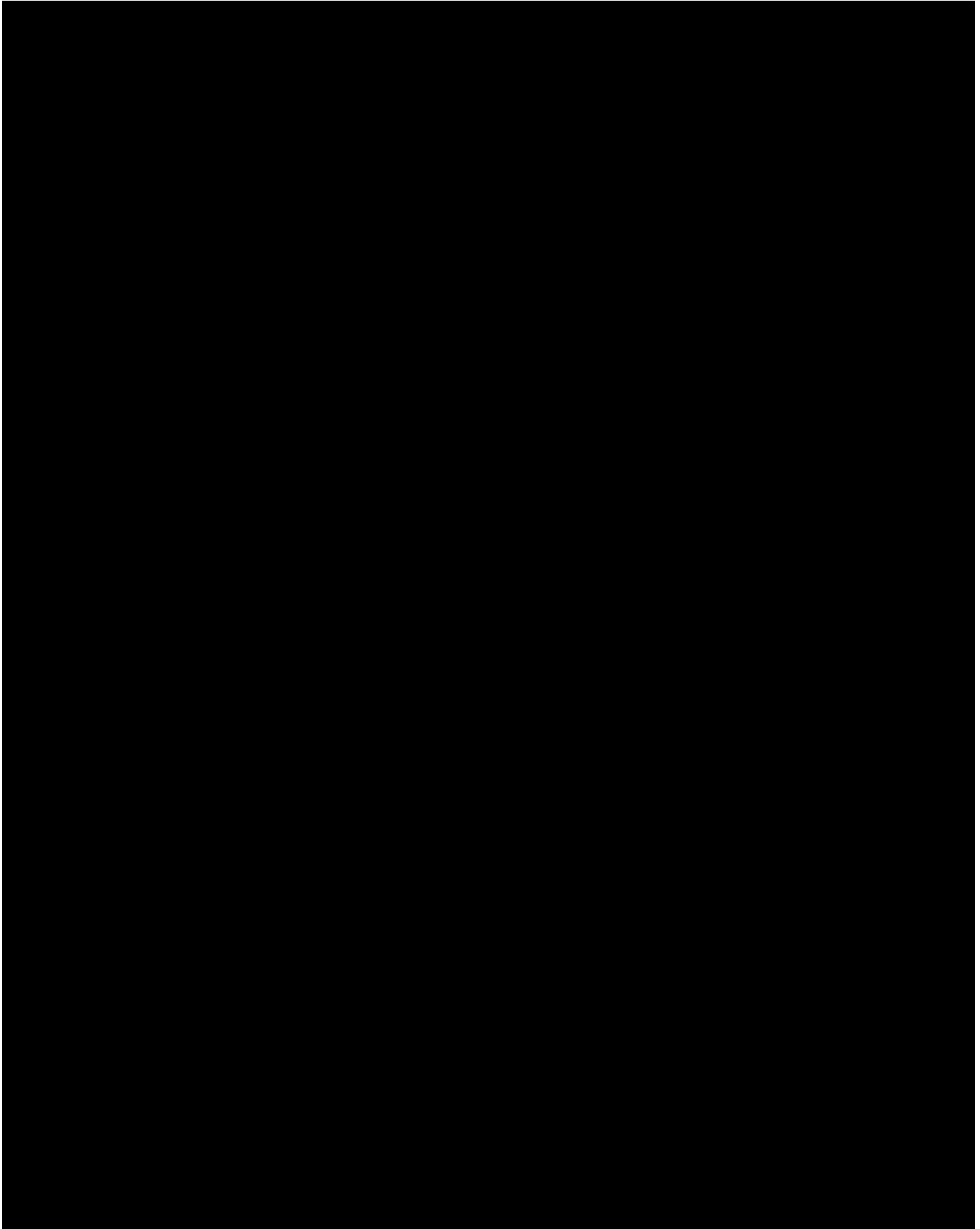
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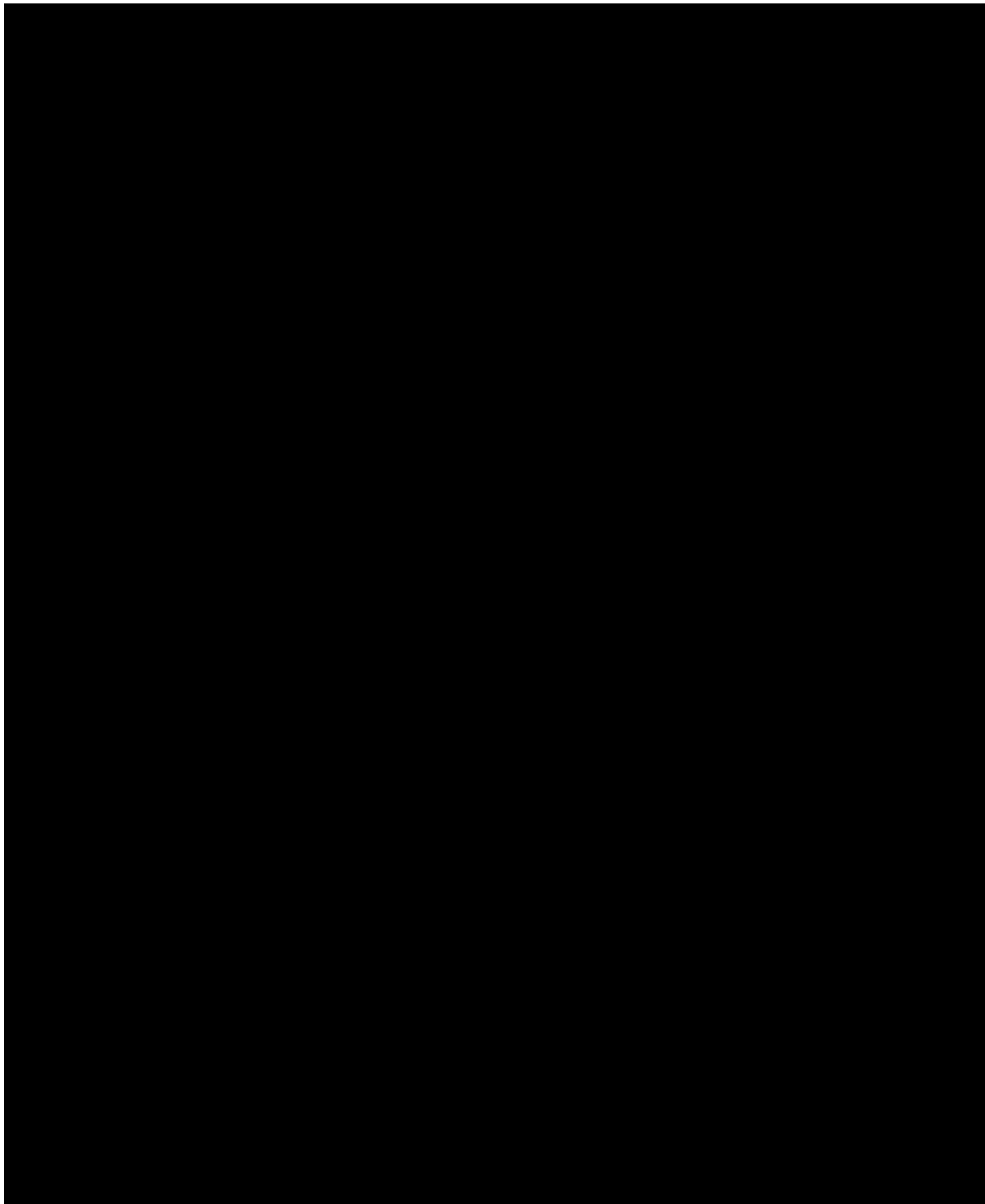
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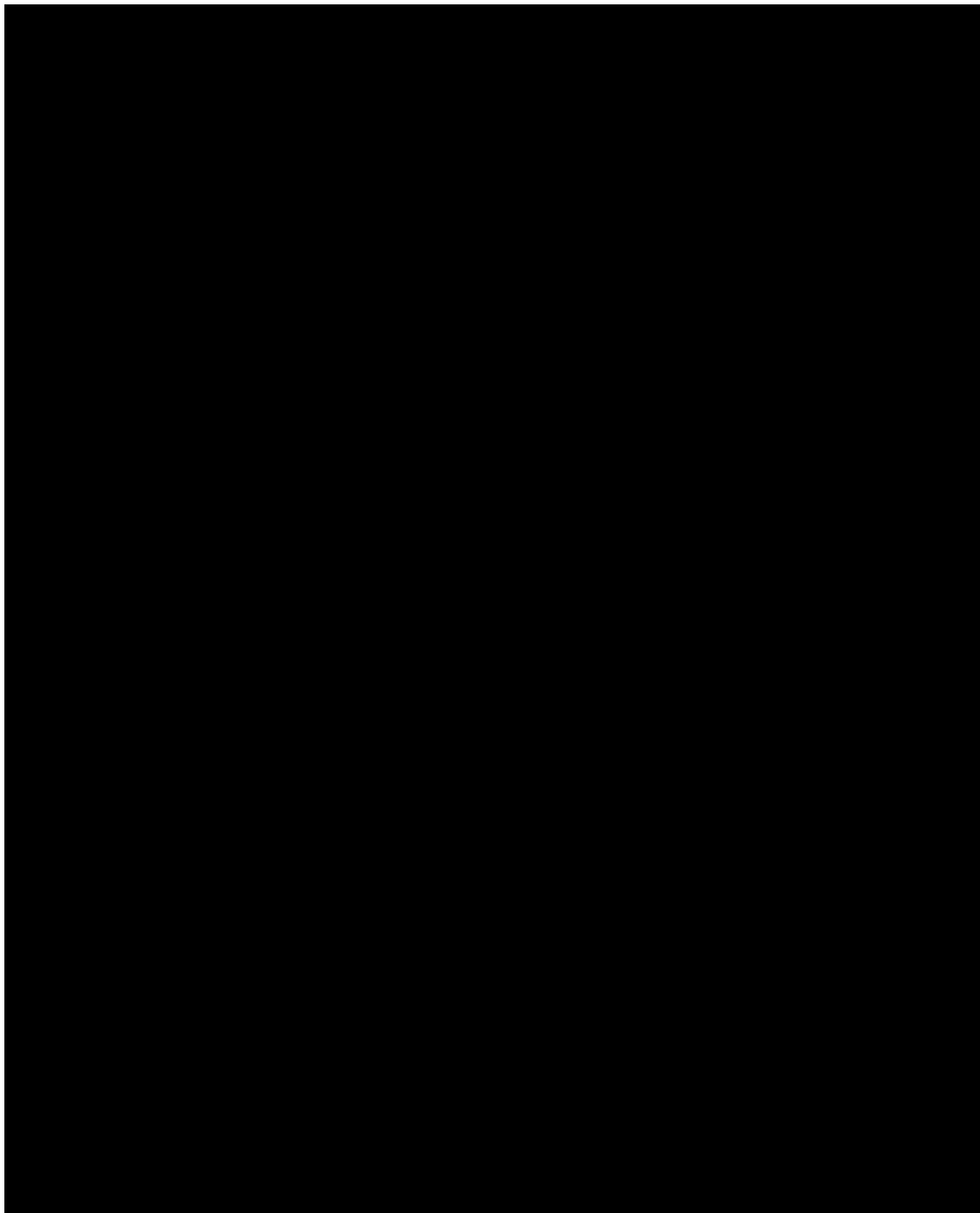
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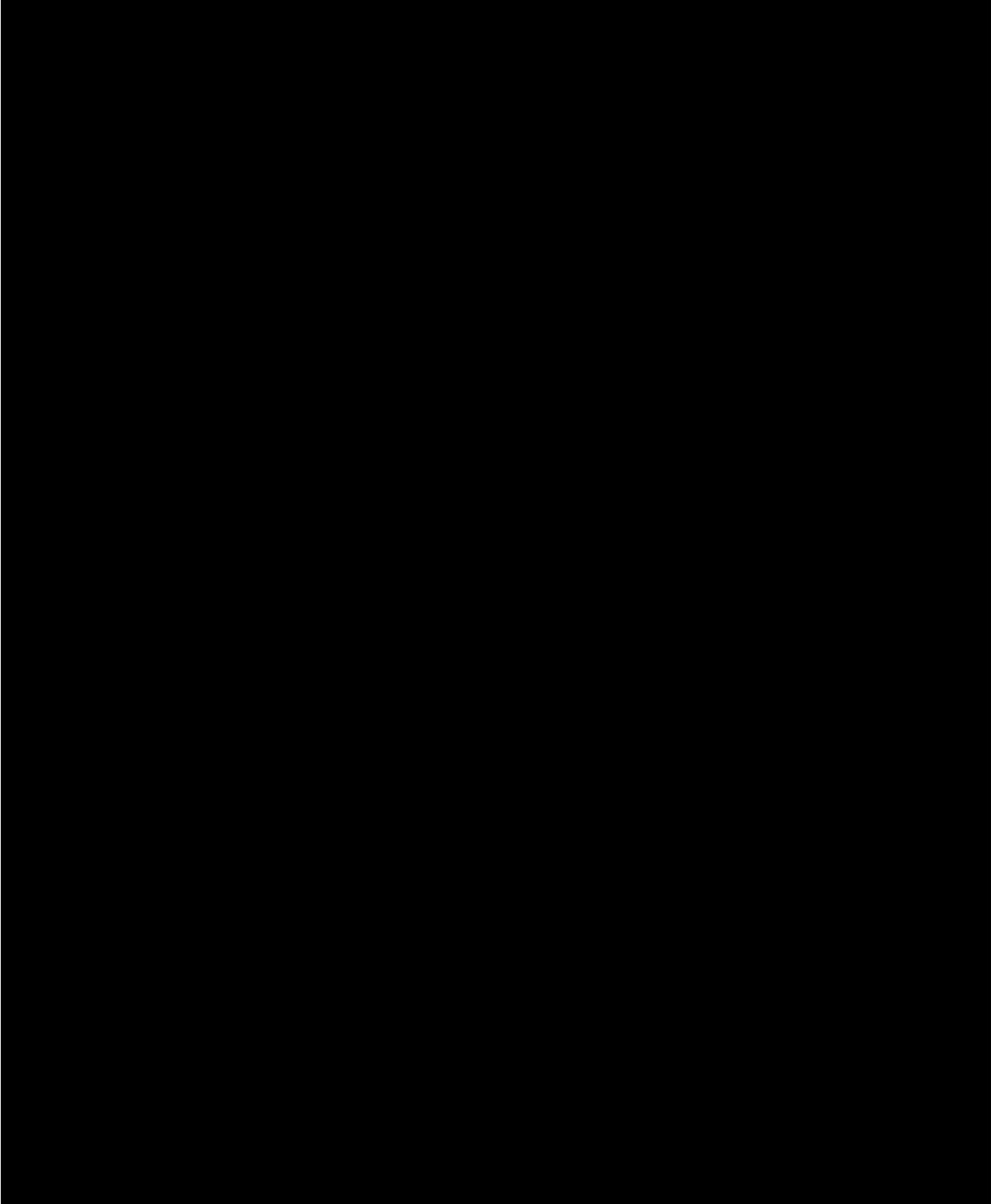
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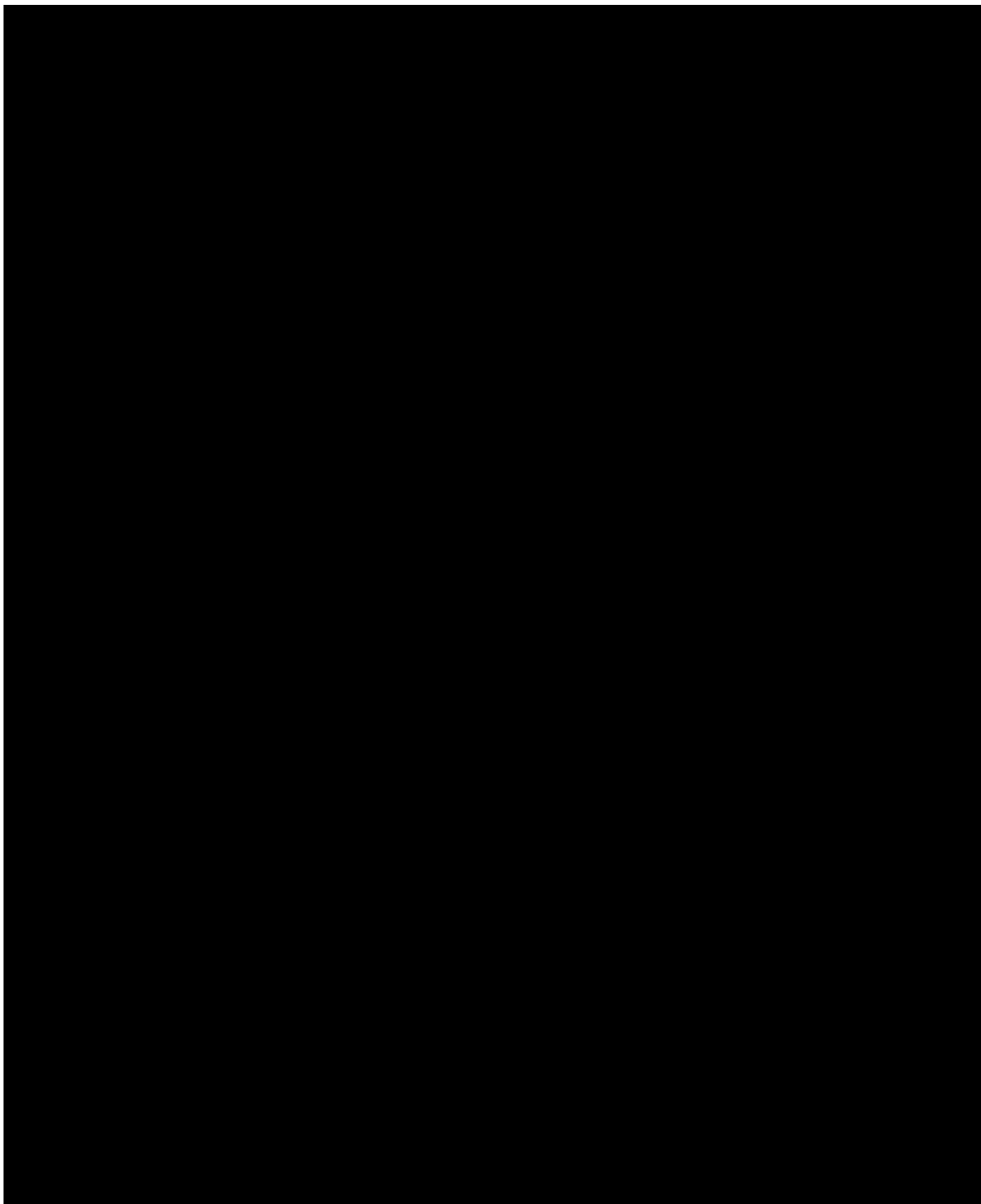
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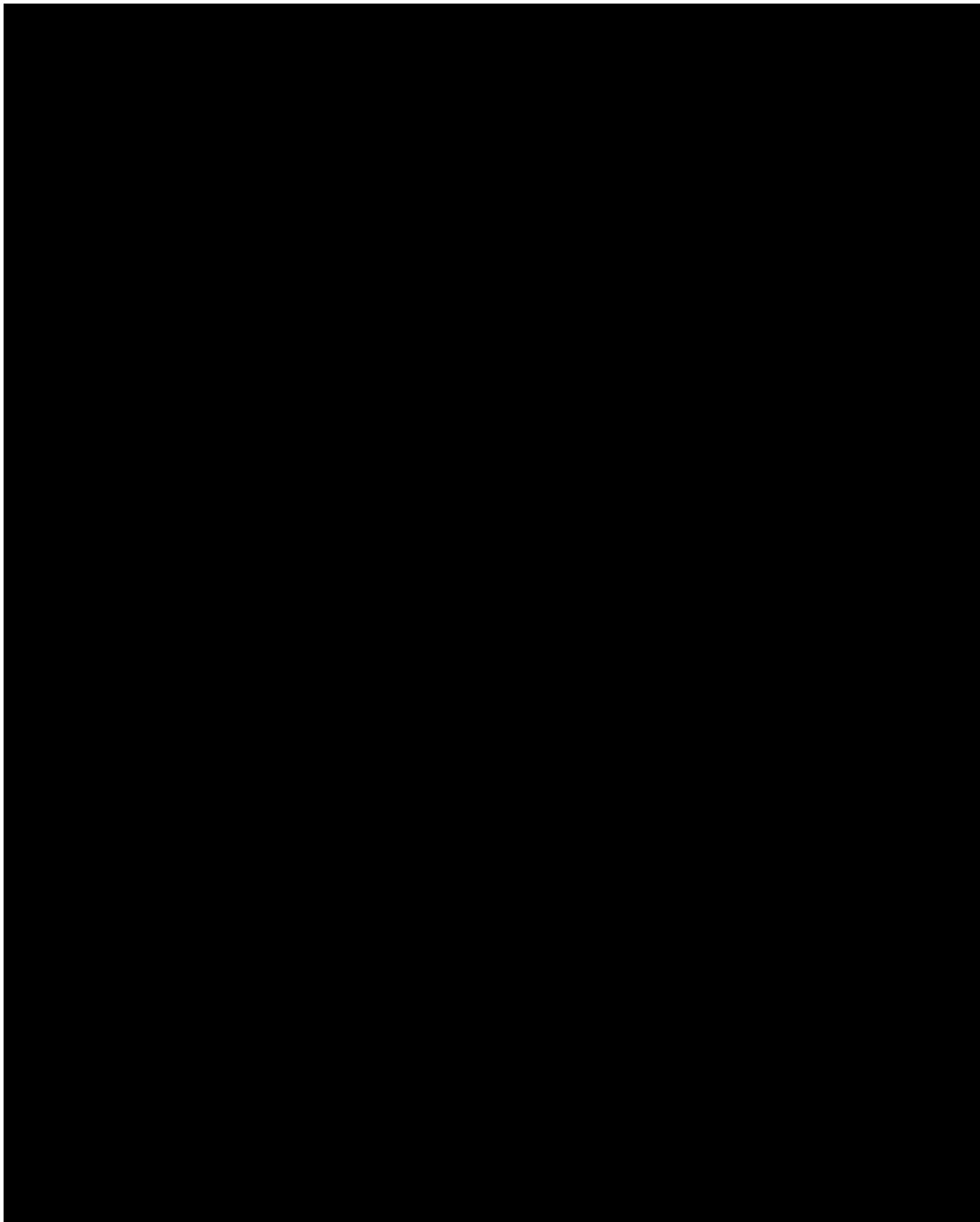
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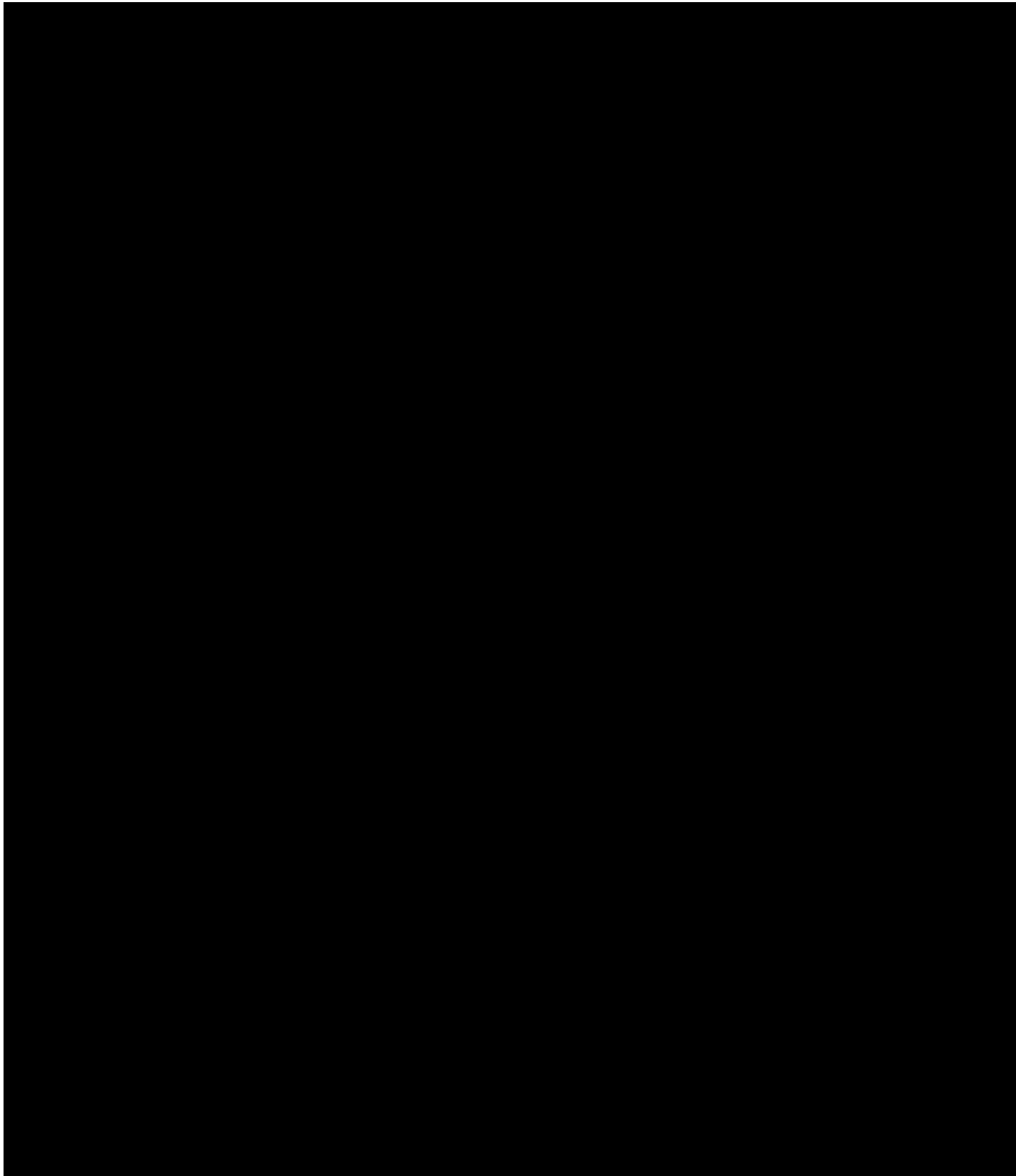
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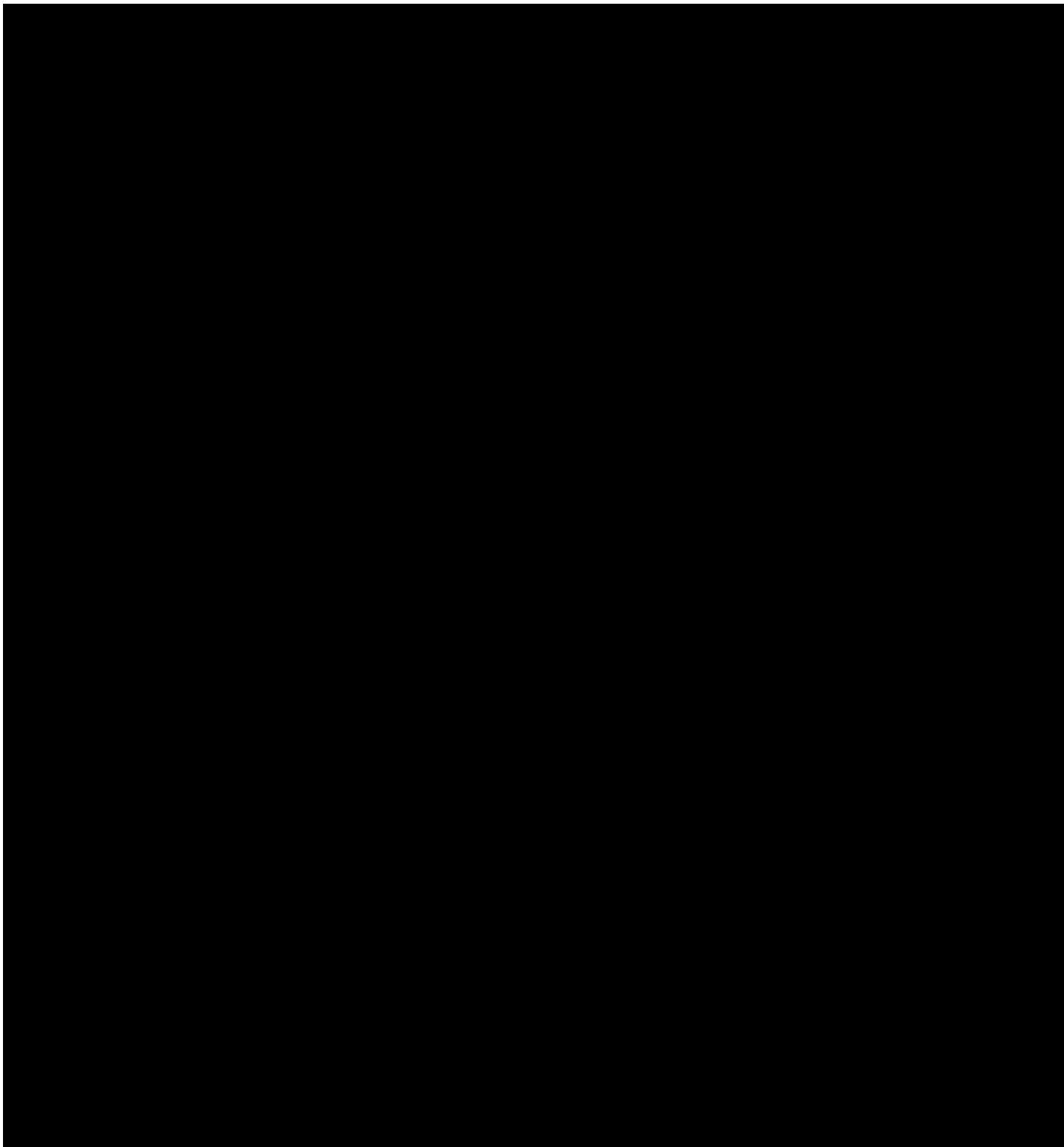
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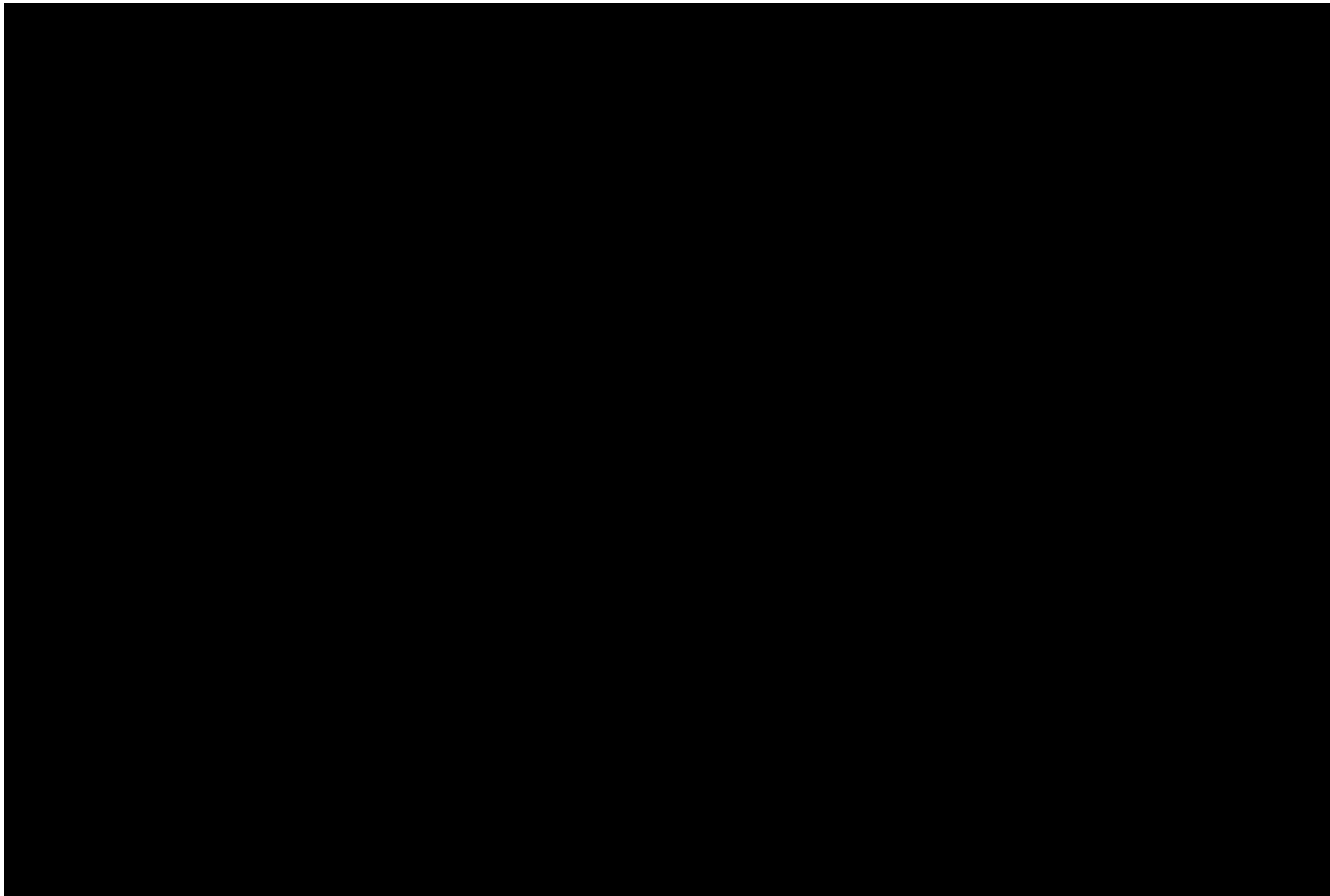
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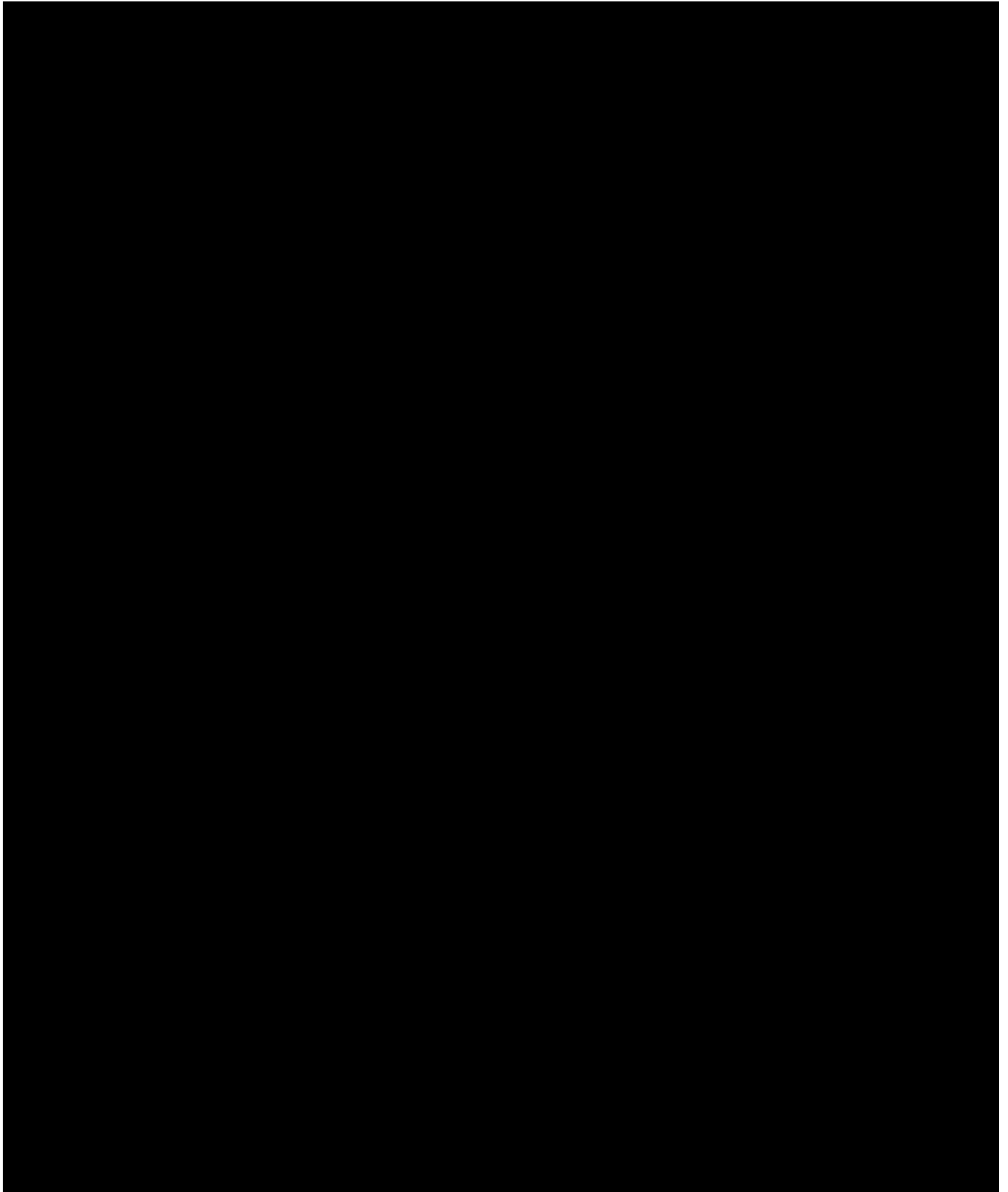
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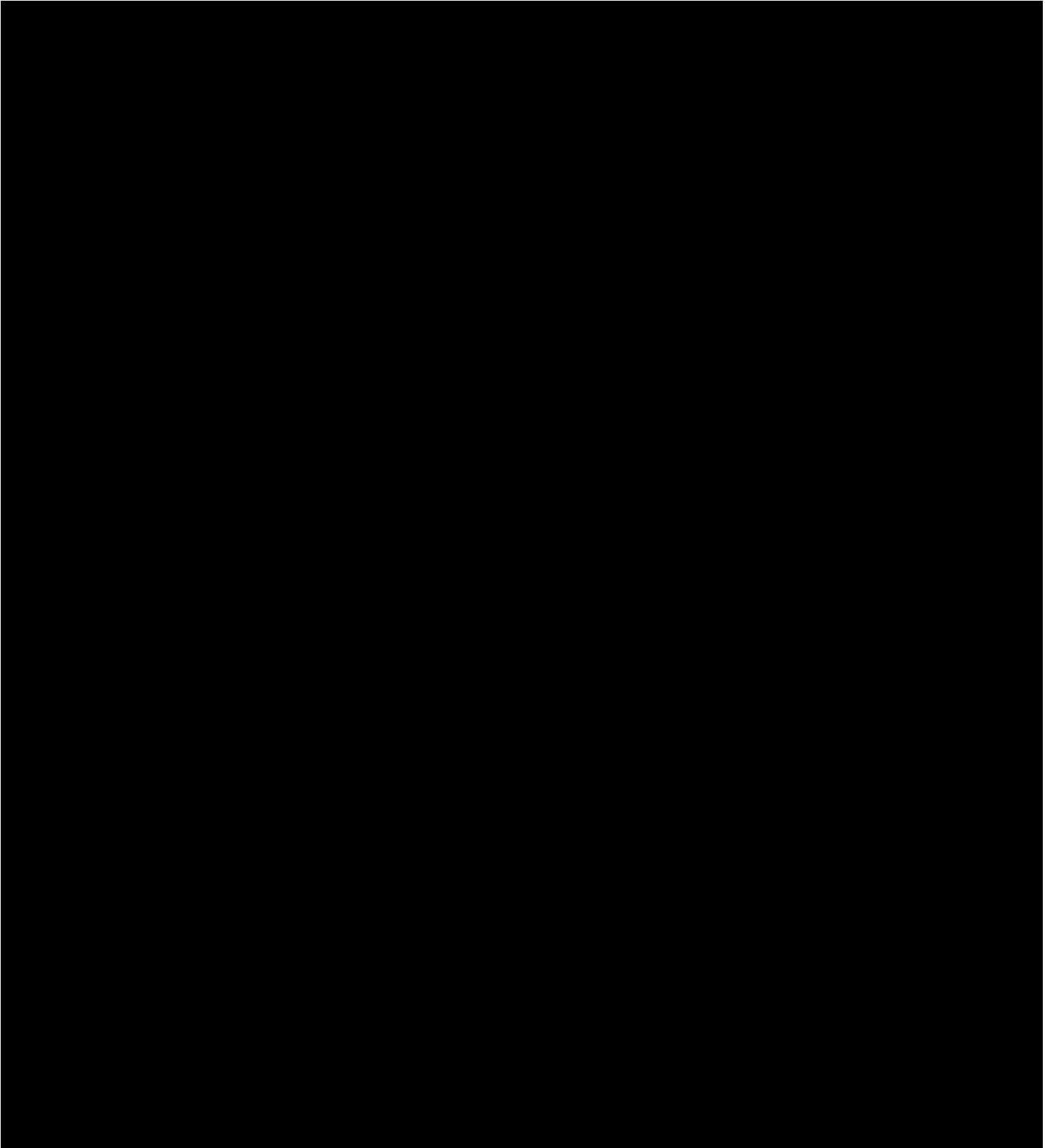
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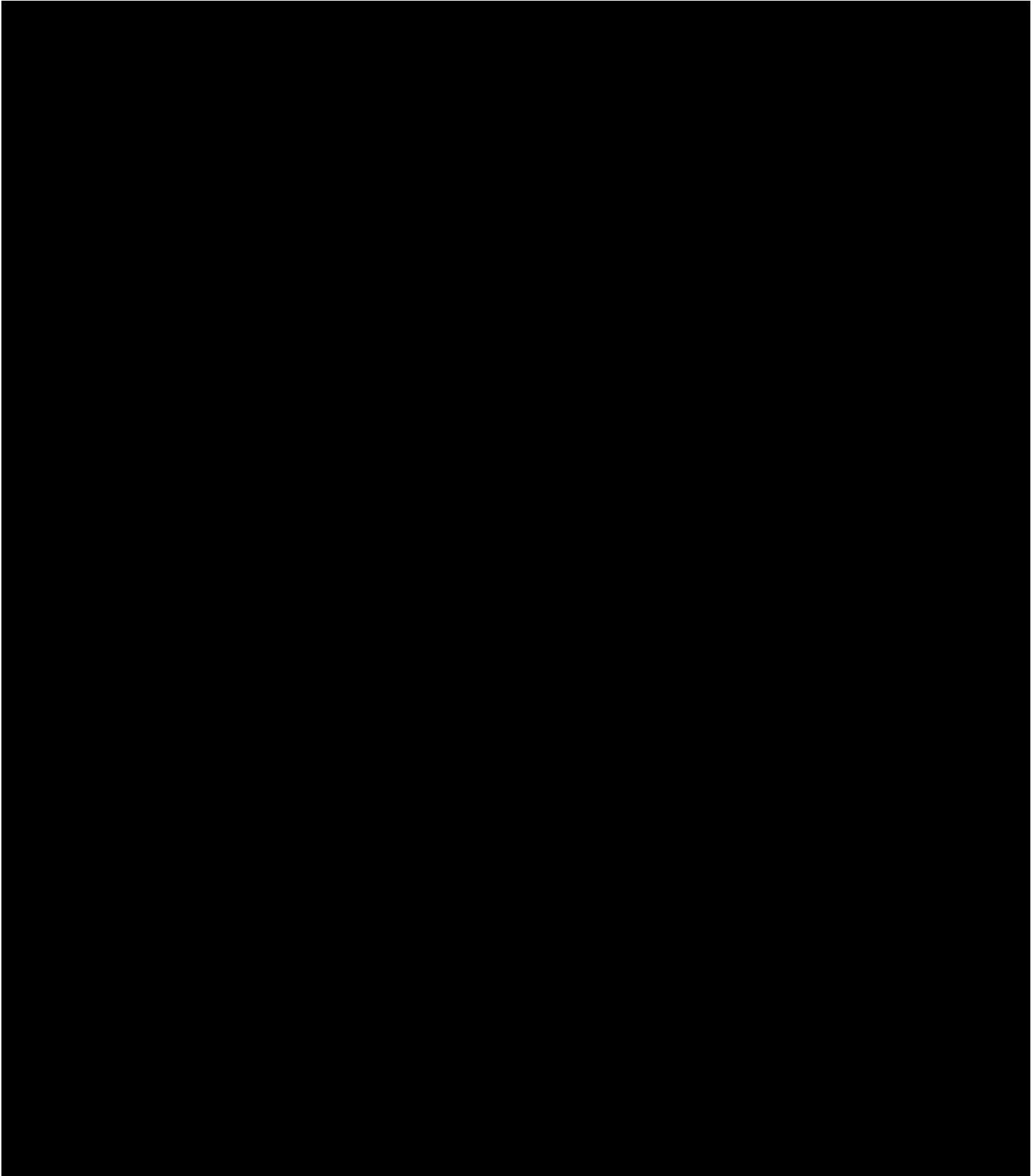
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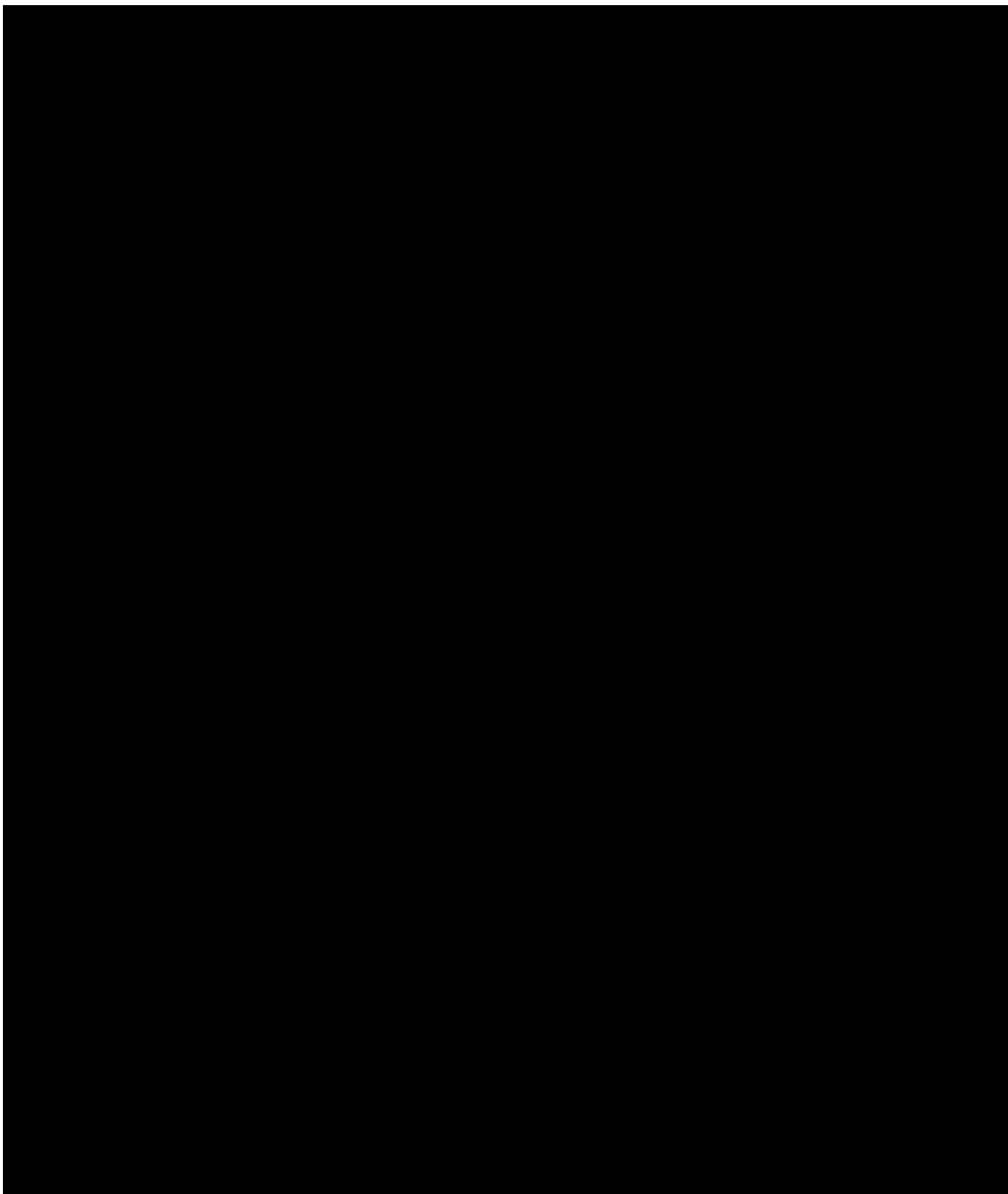
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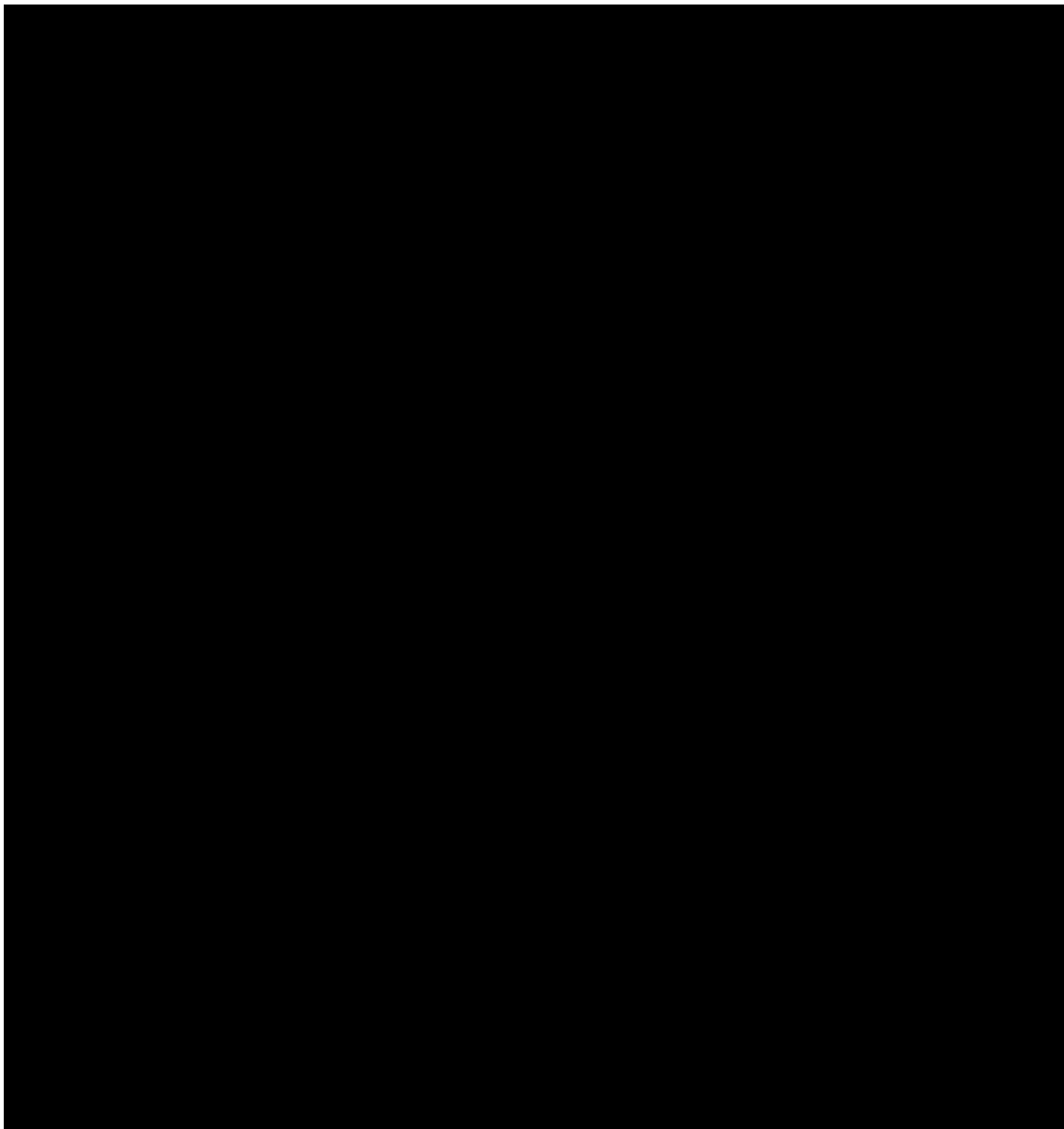
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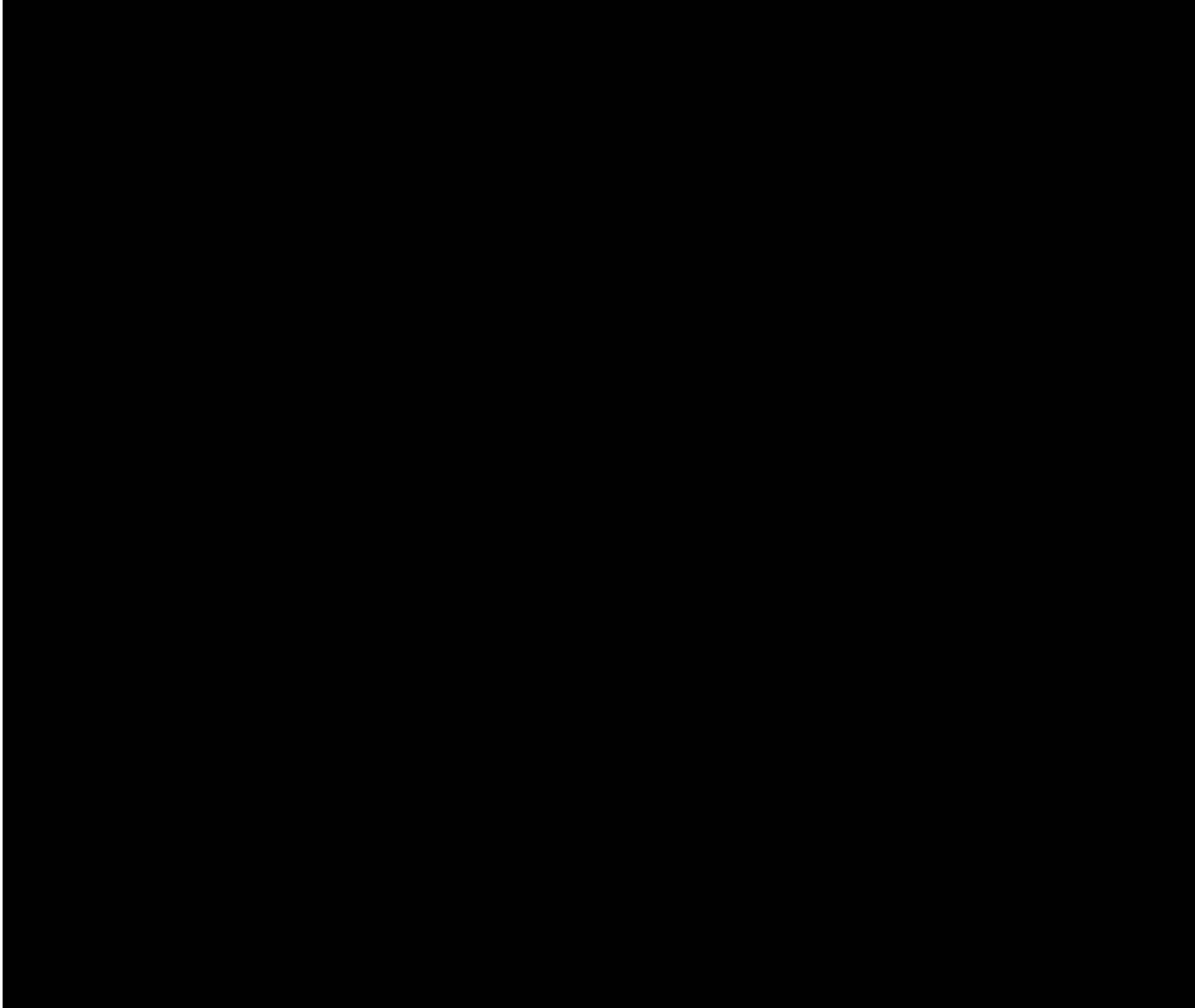
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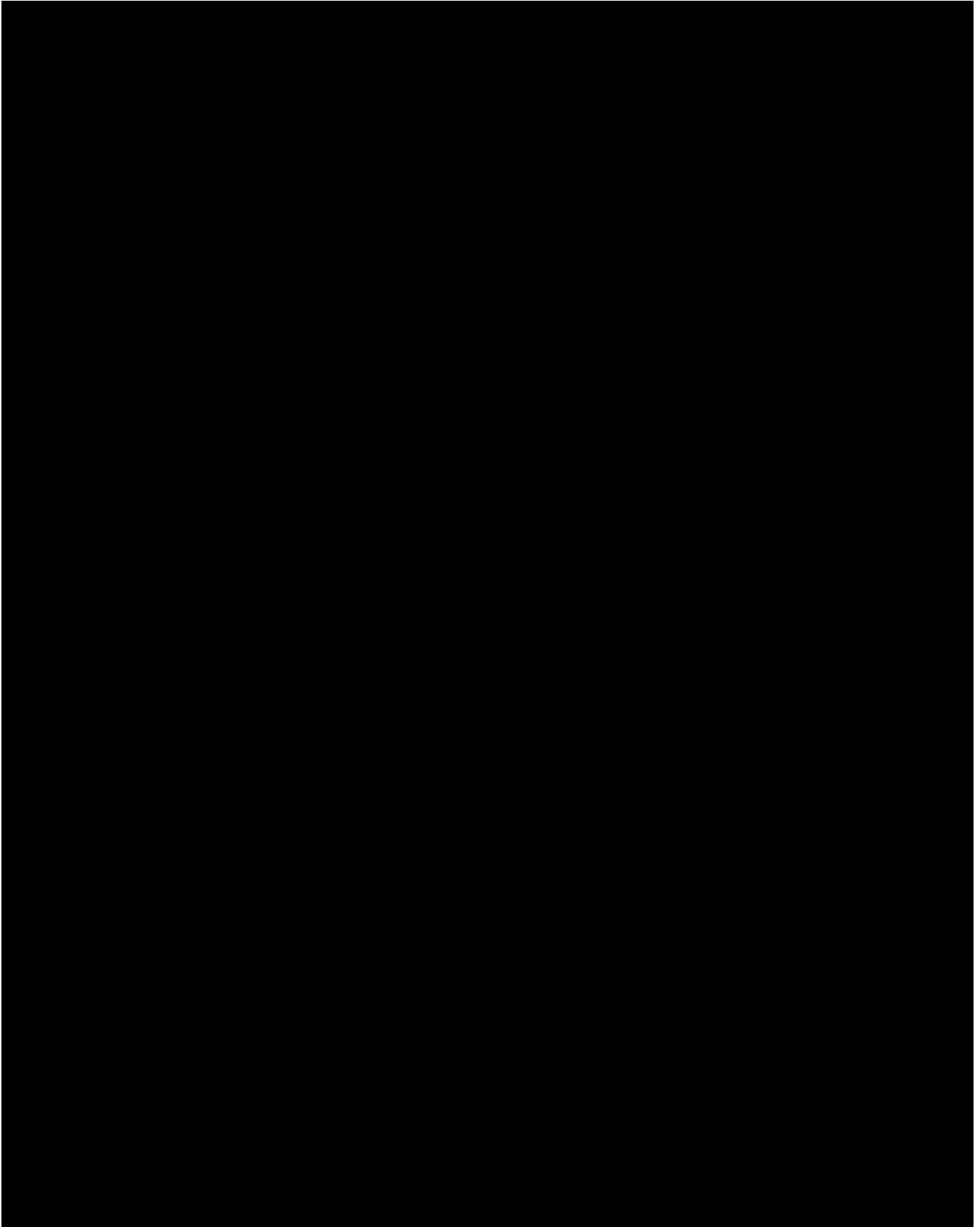
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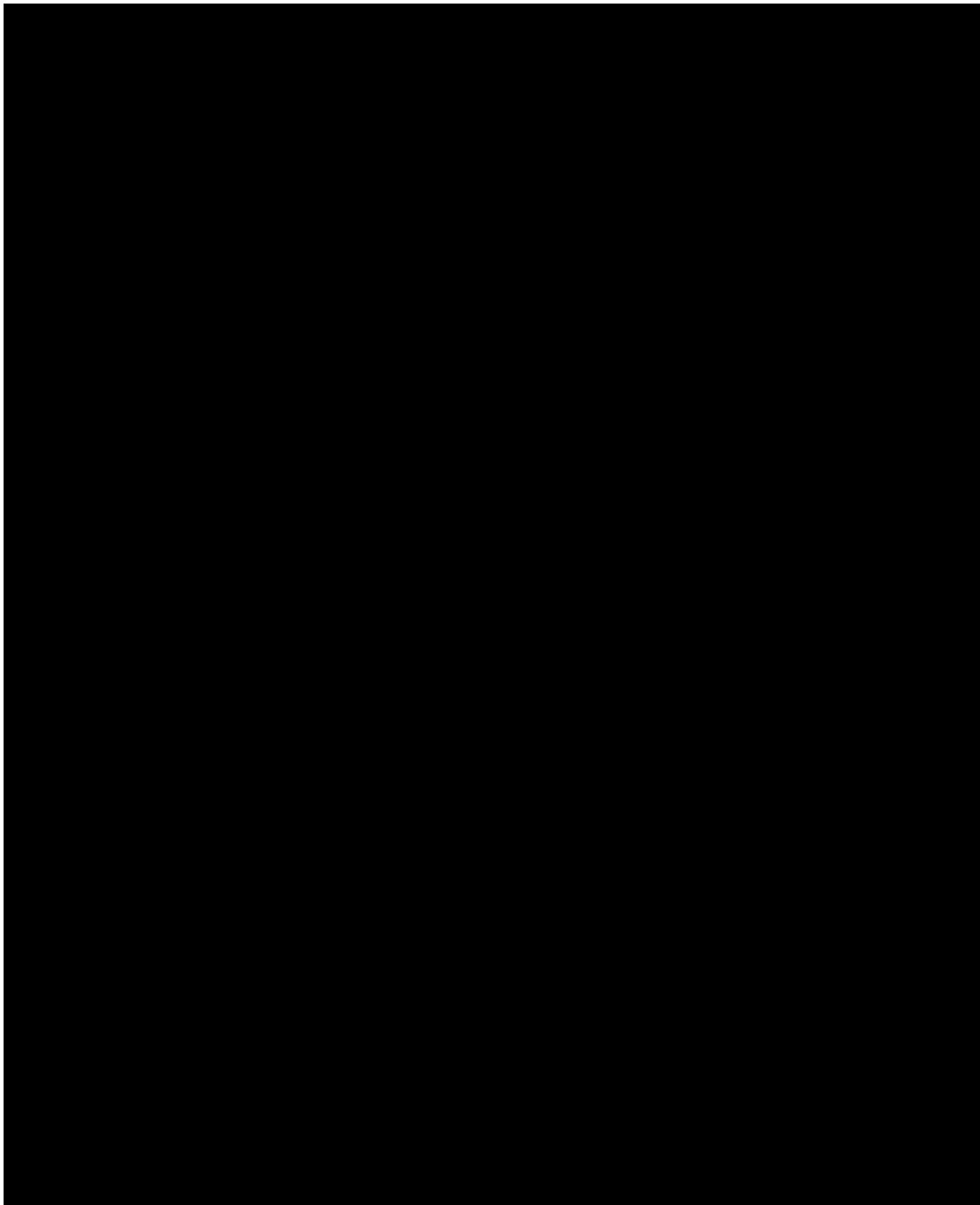
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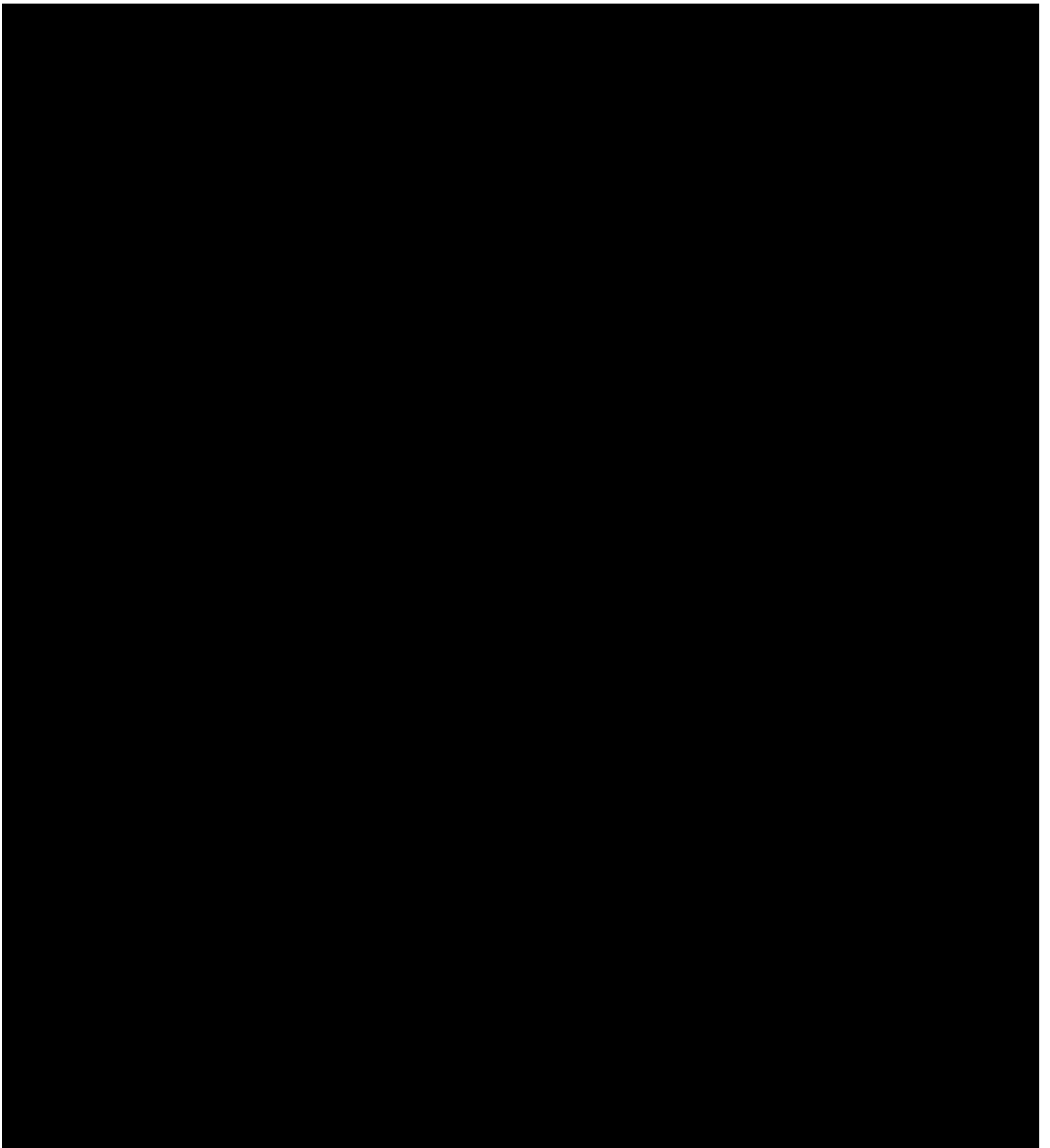
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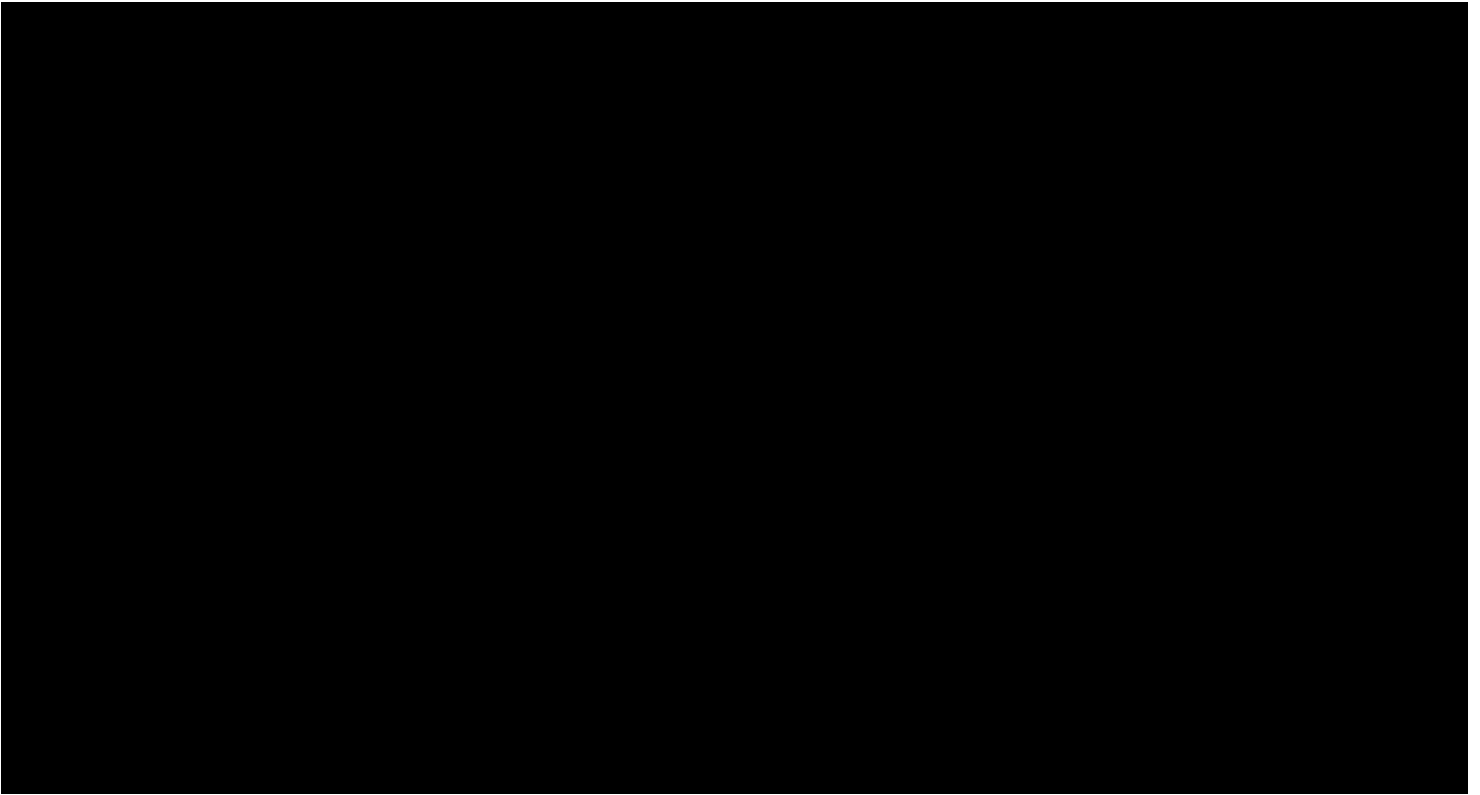
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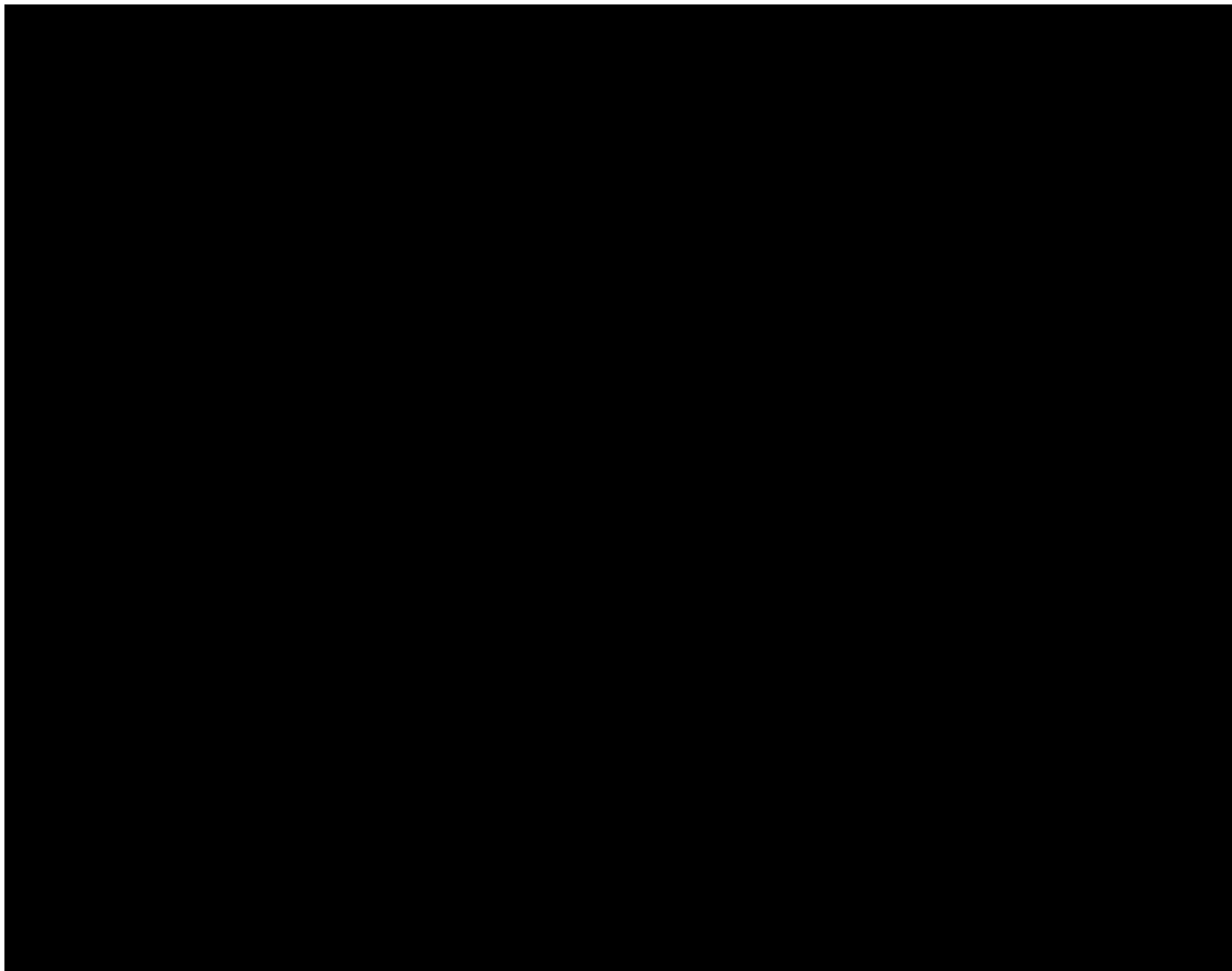
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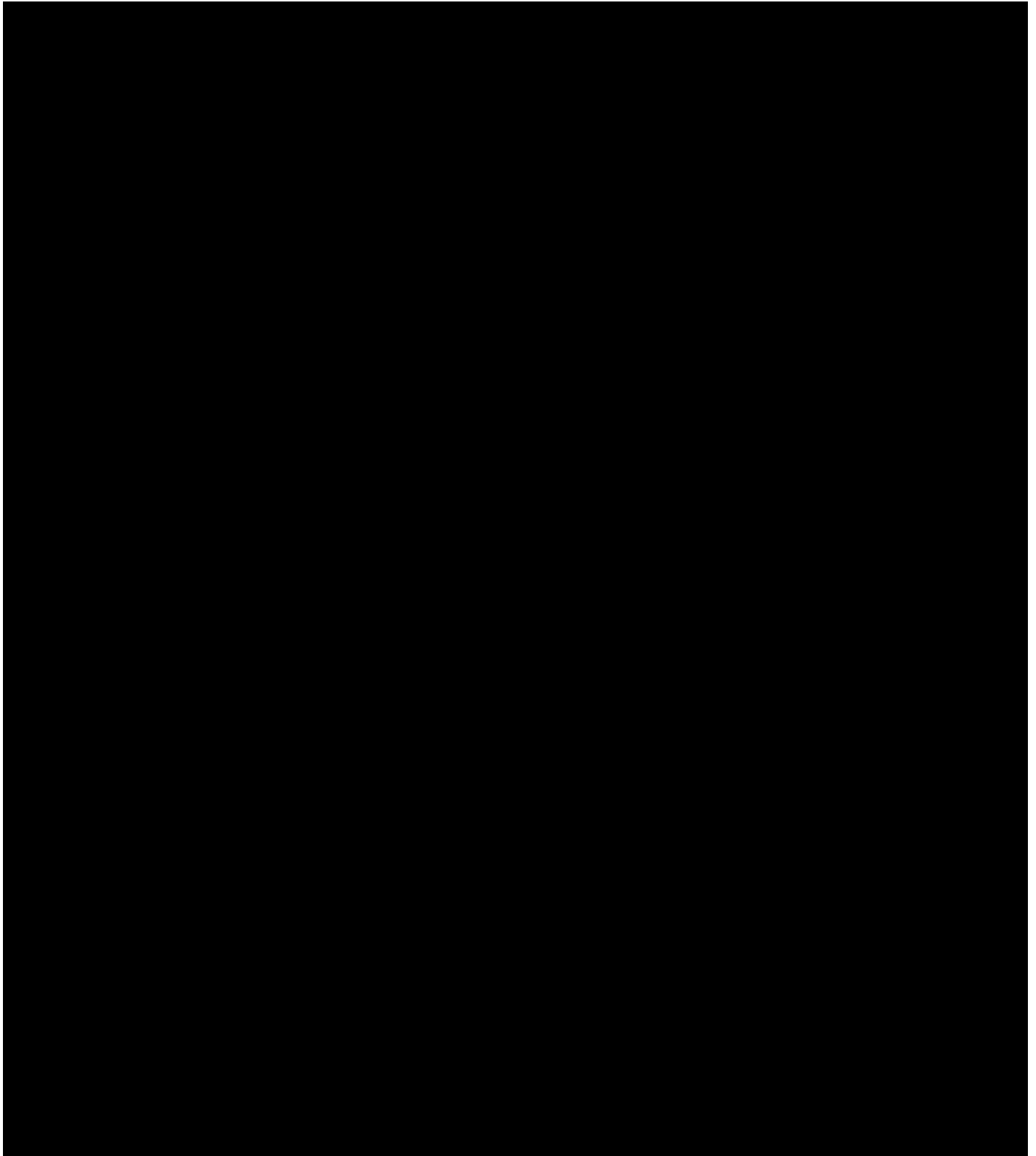


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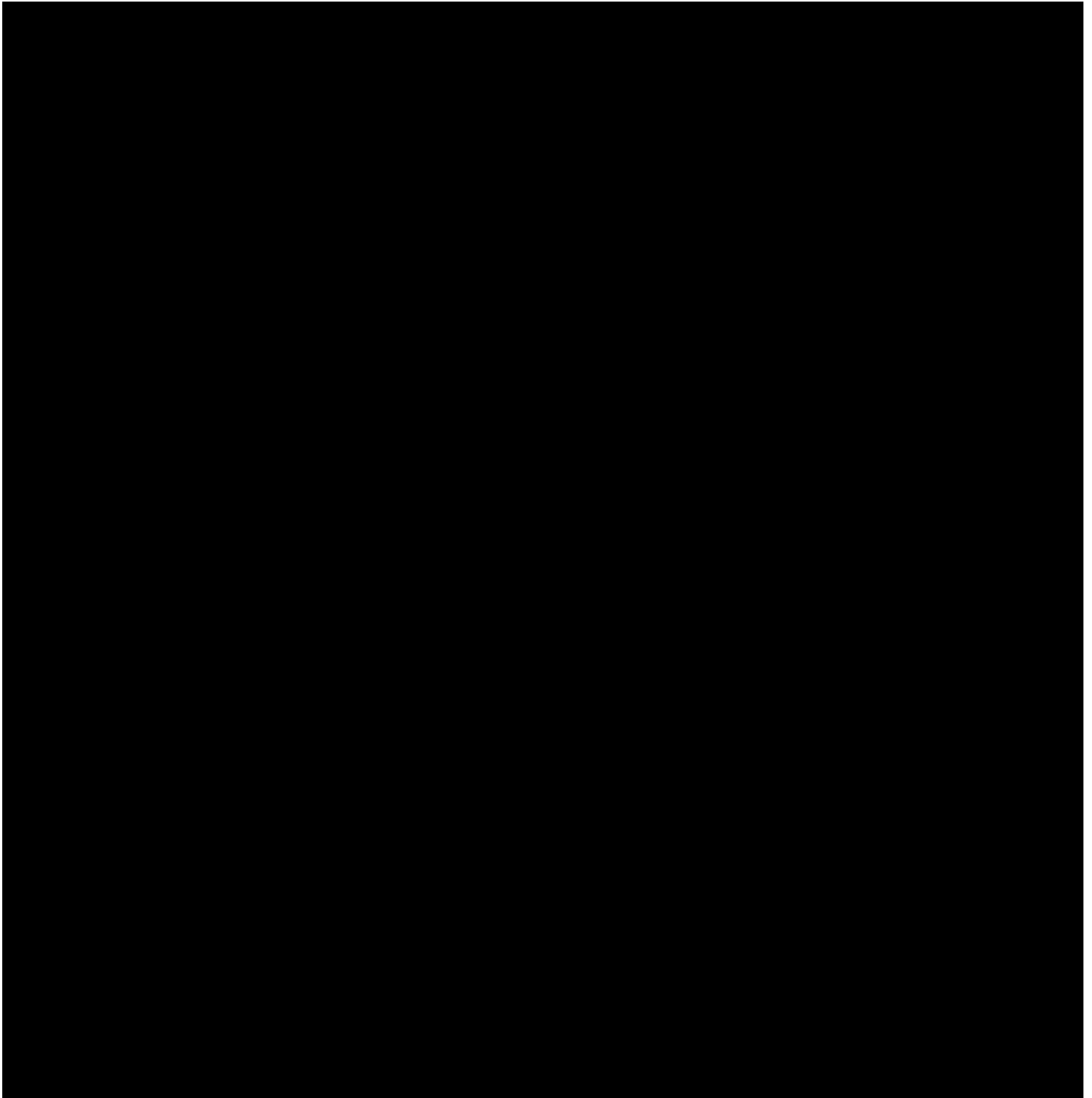




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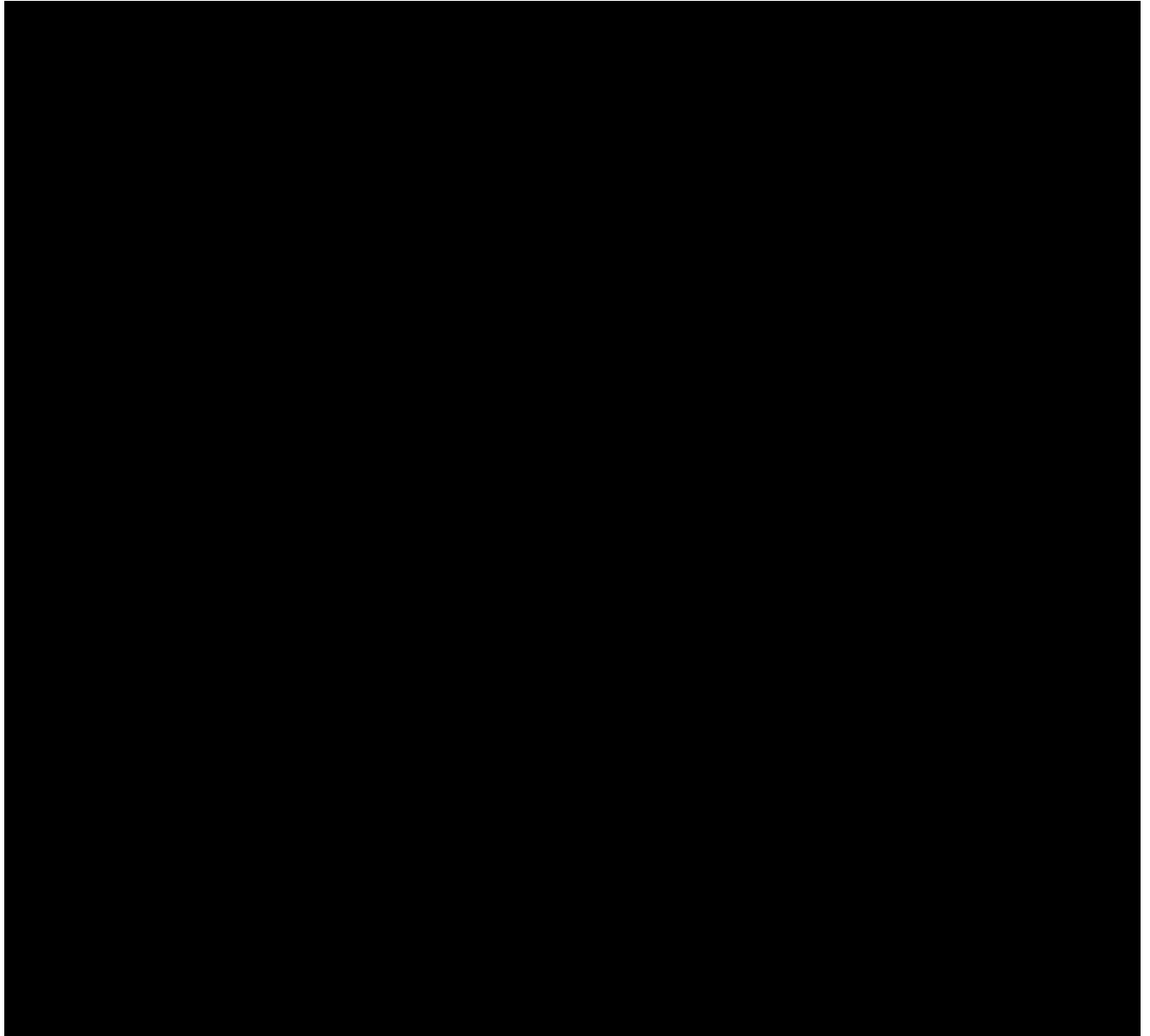
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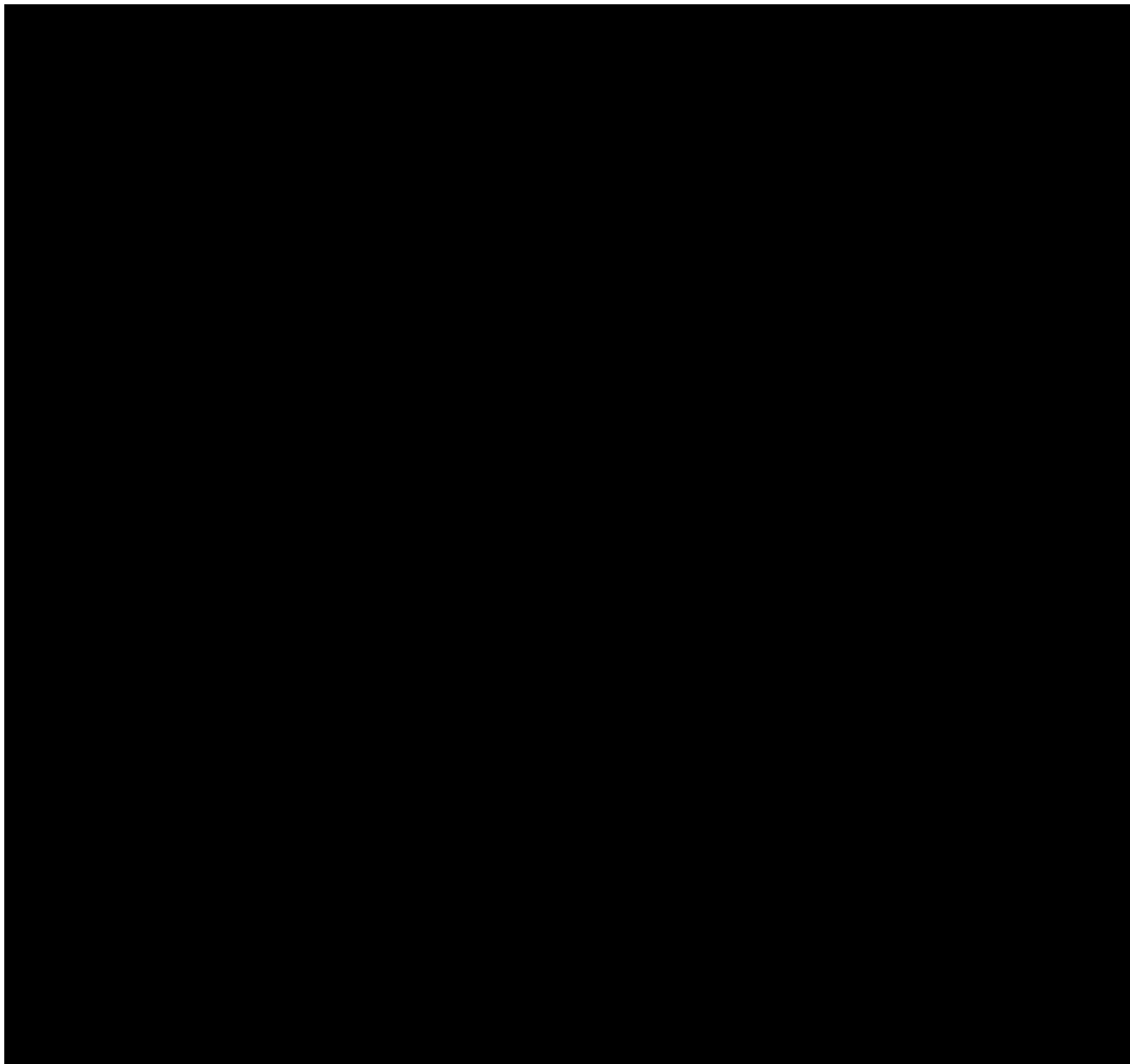
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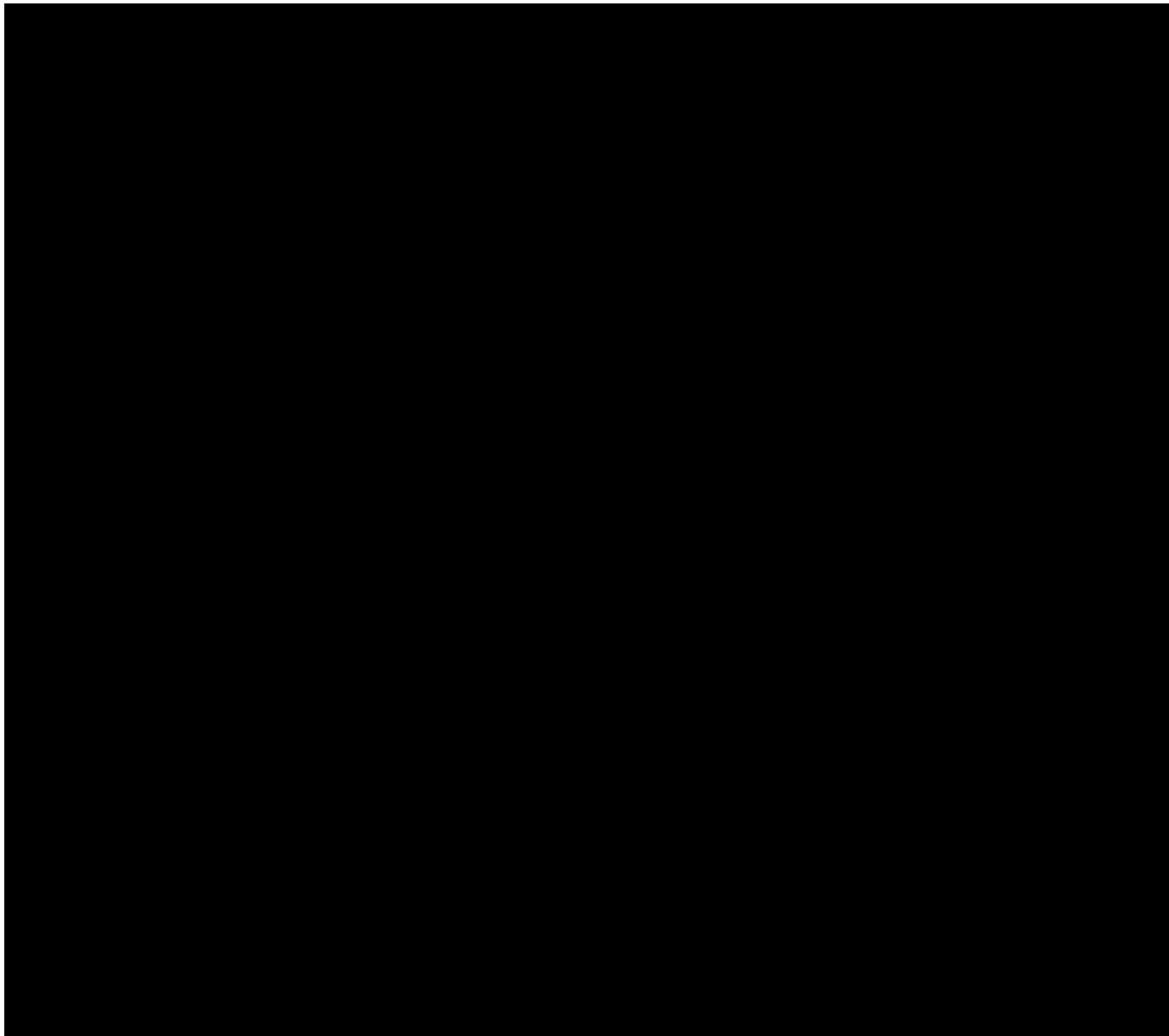
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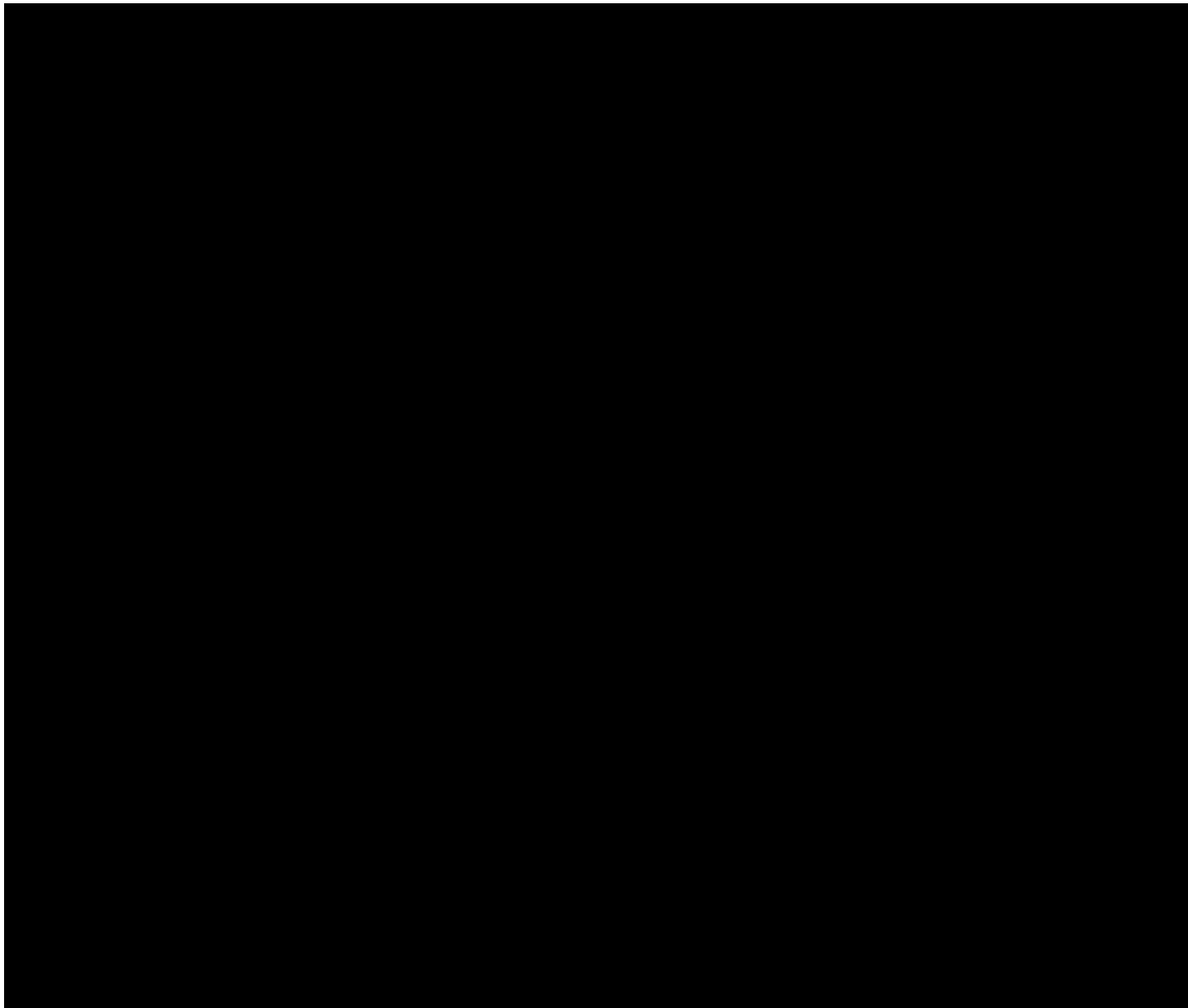
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3. Phenotypic and Ecological Characteristics

3.1 Phenotype

The phenotype information required for this MCAN is understood to refer to the expression and interaction of the genes of the organism as well as the influence of environmental factors and random variation. The interaction between these factors may be represented as genotype + environment + random variation → phenotype.

Since the inserted genetic elements in this case do not appear to possess any intrinsic hazard potential, data are being provided for the species in general based on the rationale that the modifications to the organism were not shown through a literature search to produce an effect or yield different results from the unmodified strain. For this reason, we believe it is appropriate to use the strain, *S. cerevisiae*, as a surrogate strain for gathering information and assessing the effect of the modified strain on antibiotic resistance and to tolerance to metals and pesticides. The phenotype of the MCAN strain has no significant variation from the unmodified strain except for the enhanced ability to [REDACTED]
[REDACTED]

Given that no change in the ability of the modified strain to survive and reproduce due to the disclosed modifications is anticipated, references are being provided for unmodified *S. cerevisiae* as surrogate data to evaluate the viability of the production strain in the natural environment. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED] We did not locate any papers in which such differences were observed in yeast. In the studies we reviewed:

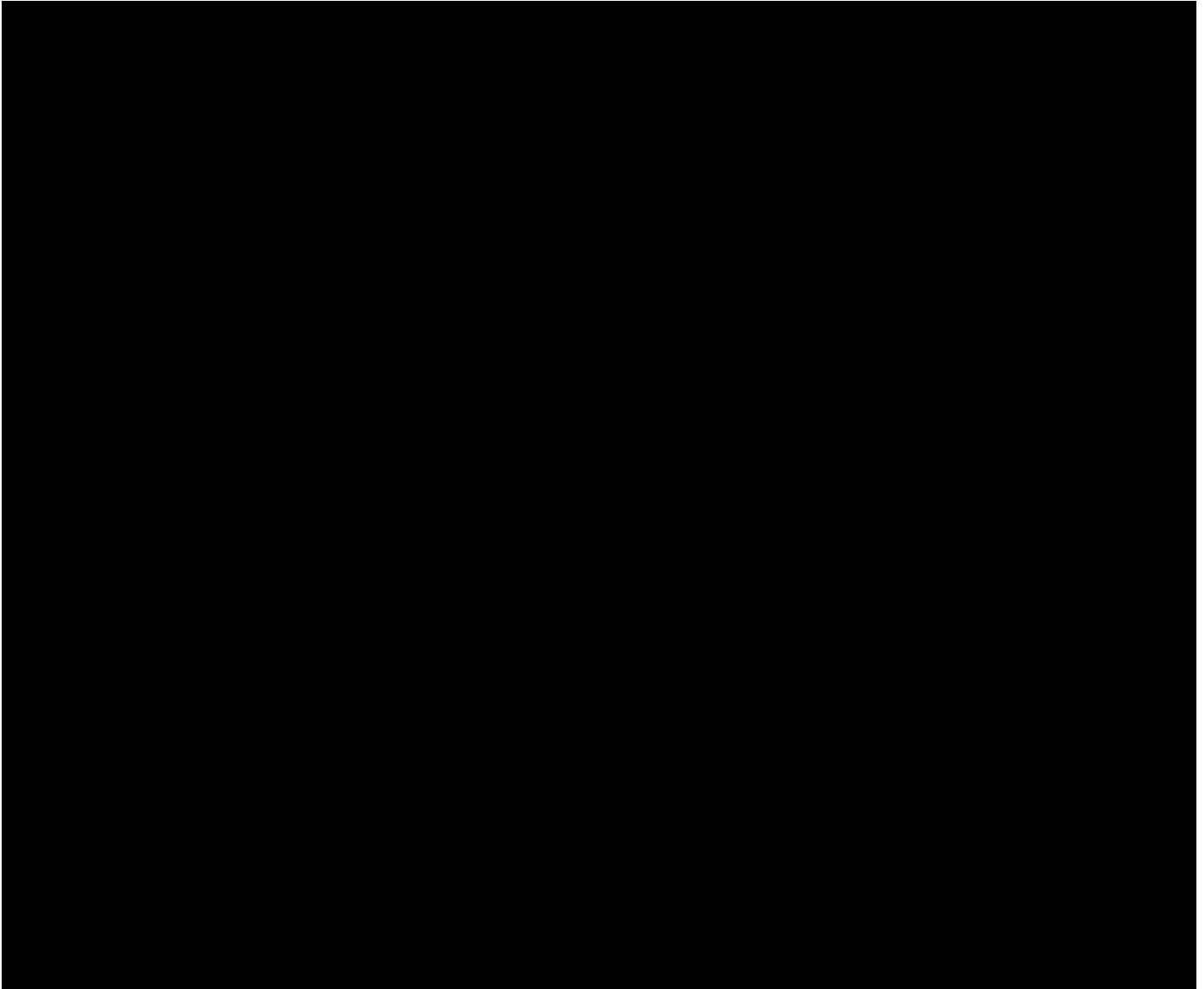
- Insertions designed to enhance the output of the yeast did not appear to be a condition that enhanced or detracted from growth and survival.

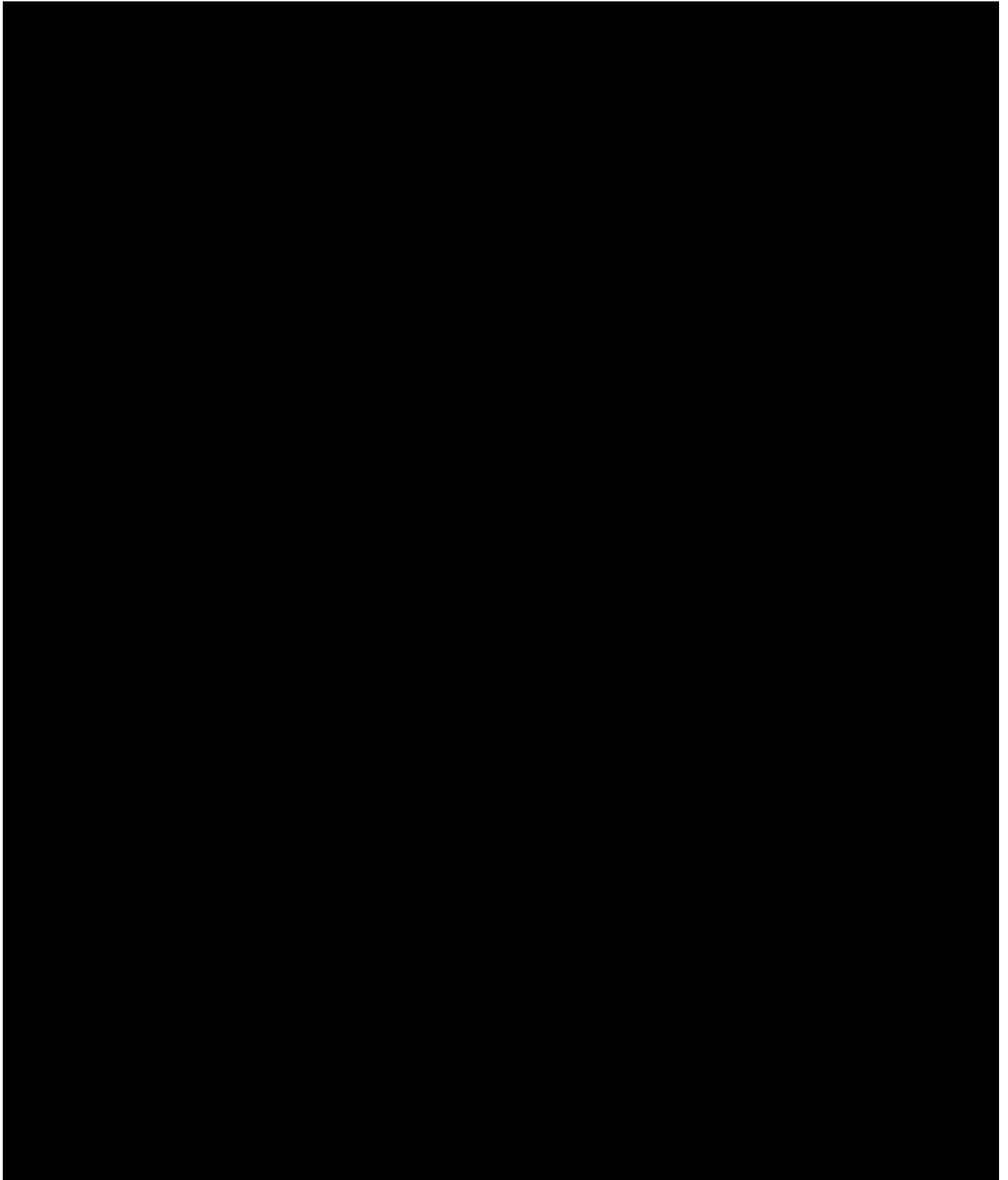
- Normal environmental conditions (room temperature, neutral pH) did not affect comparative growth and survival.
- No differences in growth and survival were observed under the following conditions: a simulated vineyard environment, a soil/water suspension, a growth medium/soil environment, wastewater, and soil with a water content of 7.2% and a pH of 6.5.

The primary condition identified as necessary for growth of modified or unmodified yeast is a nutrient rich environment.

3.2 Habitat, Geological Distribution and Source

3.2.1 Donor Organisms

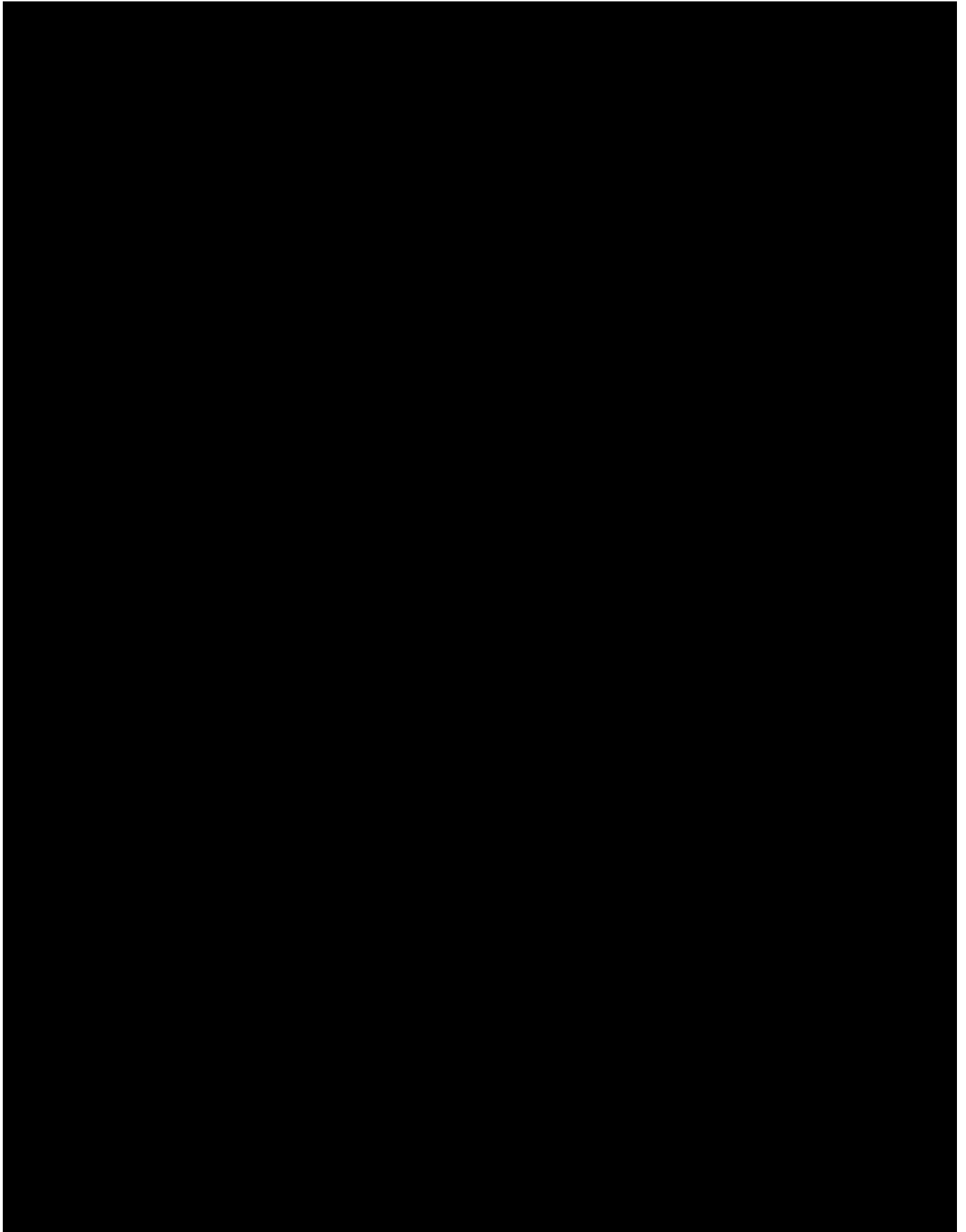




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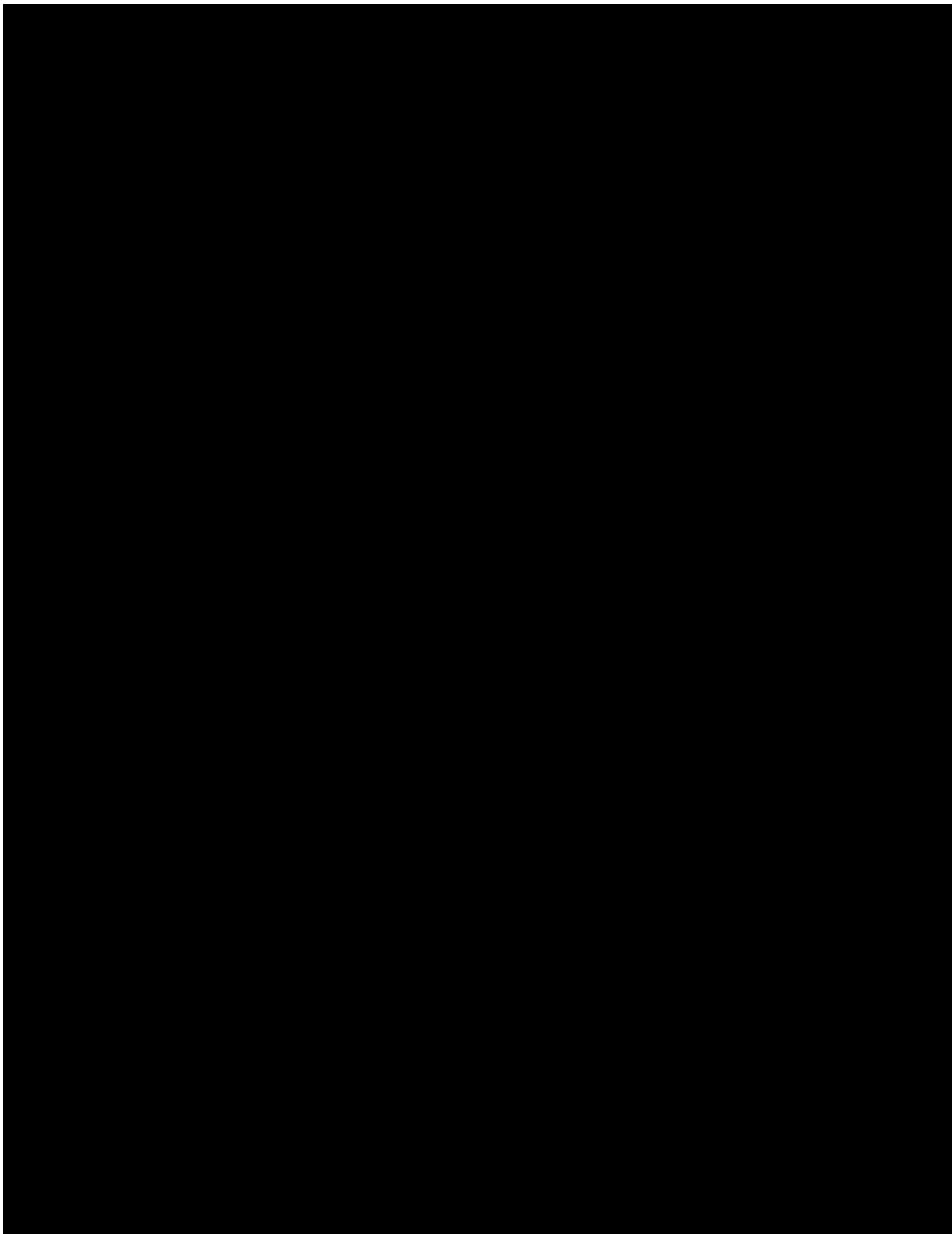
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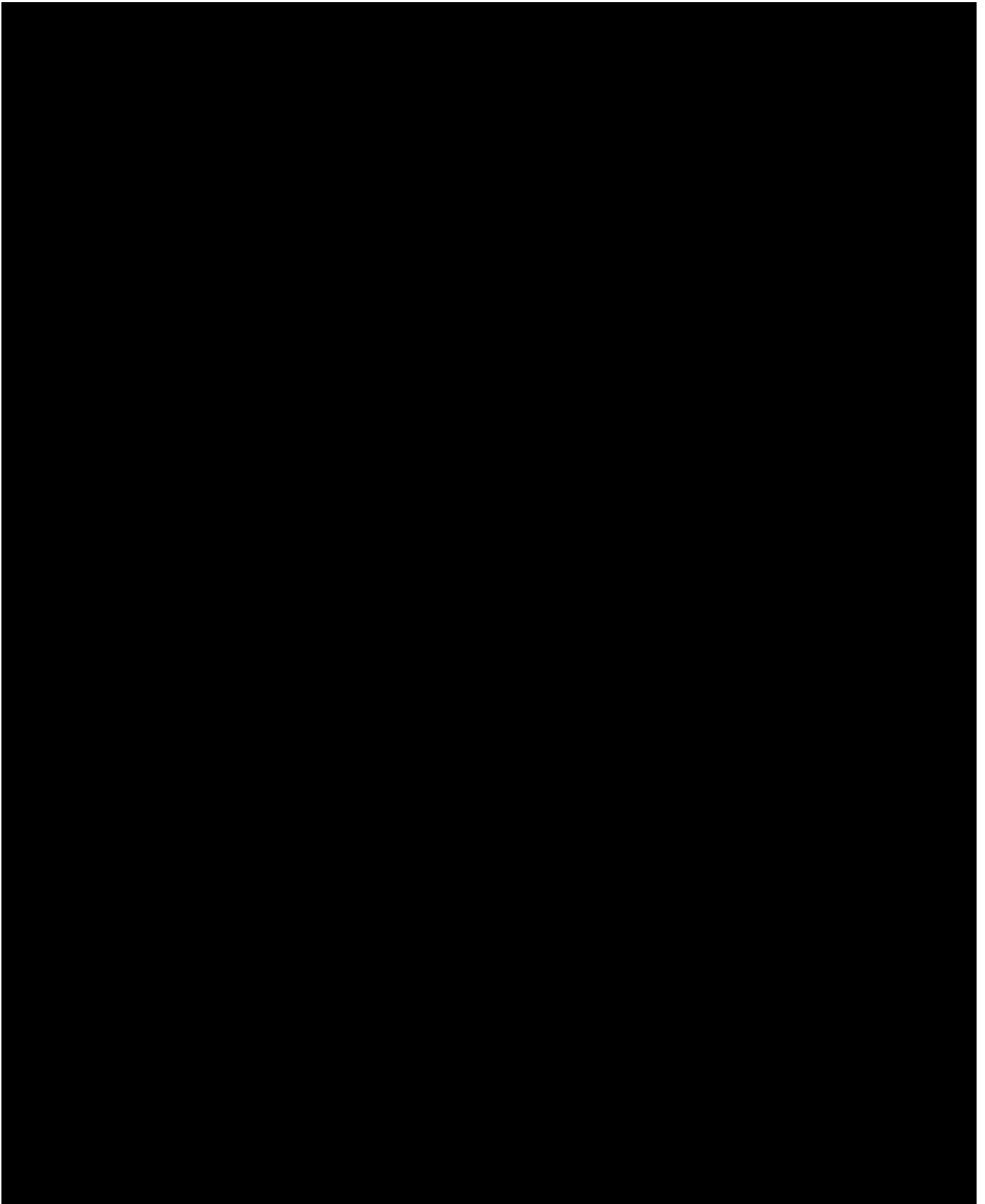
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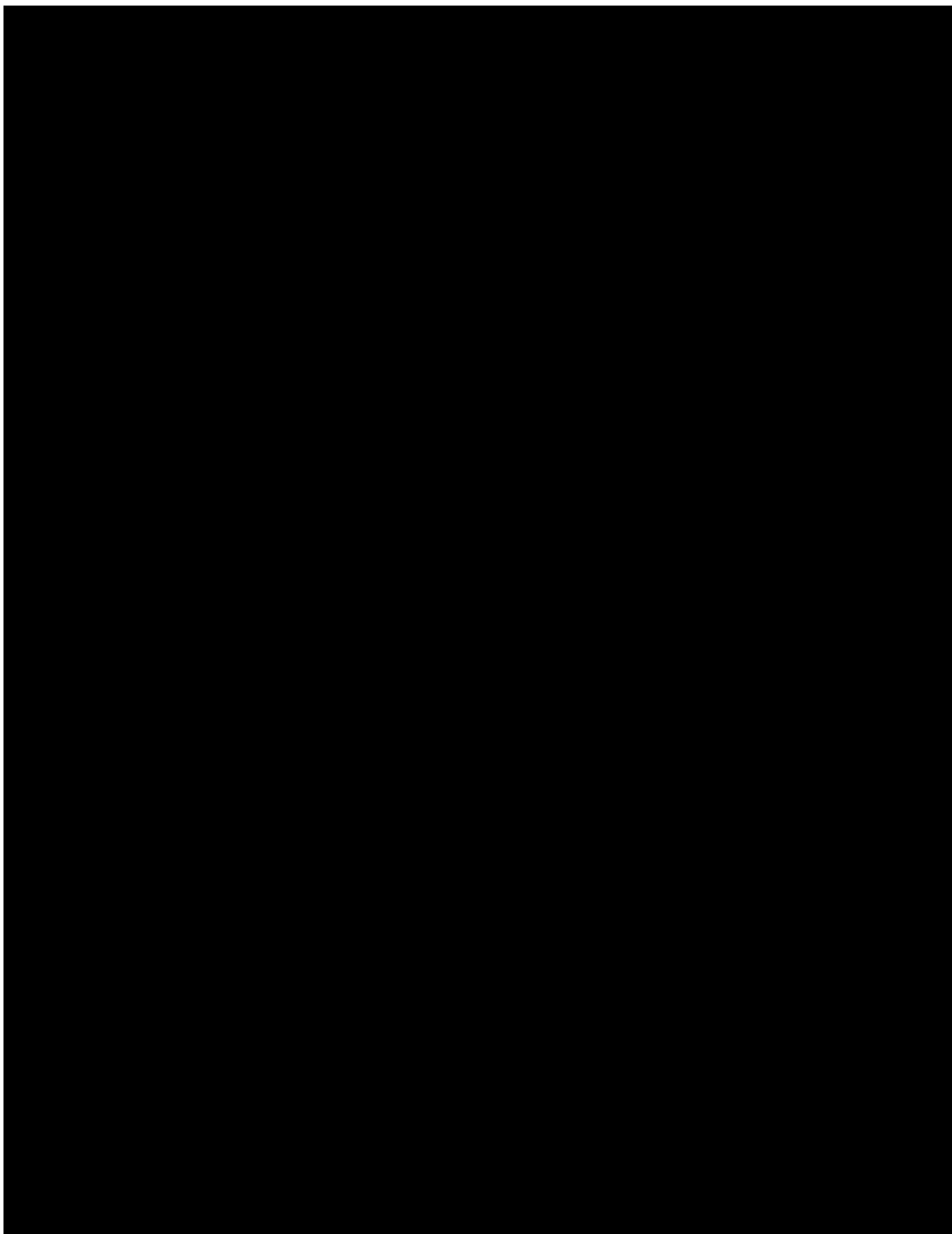
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3.2.2 Host Organism

S. cerevisiae is widely distributed in a variety of environments, found on each continent, and therefore can be described as ubiquitous.⁷⁶ EPA in its final risk assessment describes *S. cerevisiae* as a being present in fruits and vegetables and ubiquitous in nature.⁷⁷ In addition to reports of *S. cerevisiae* near areas with human activities, *S. cerevisiae* has been documented in uncultivated woodland areas in tree fluxes, tree bark, and soil.⁷⁸

3.3 Survival and Dissemination

It is presumed that the production strain may reproduce asexually through budding; however, the introduced [REDACTED]

[REDACTED] do not enhance the ability of the strain to reproduce in this manner or to exist in habitats different than that of the parental strain [REDACTED]

⁷⁶ Liti G, Barton D.B.H., Louis E.J. (2006). Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. *Genetics* 174. 839-850.

⁷⁷ U.S. EPA. (1997). *Saccharomyces cerevisiae* Final Risk Assessment: Attachment I--Final Risk Assessment of *Escherichia Coli* K-12 Derivatives. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Biotechnology Program under the Toxic Substances Control Act (TSCA).

⁷⁸ Sniegowski P.D., Dombrowski P.G., Fingerman E. (2002). *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* coexist in a natural woodland site in North America and display different levels of reproductive isolation from European conspecifics. *FEMS Yeast Research* 1. 299-306.

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

The scientific literature on *S. cerevisiae* and its viability in the natural environment is extensive. For example, modified and unmodified *S. cerevisiae* were sprayed on leaves, grapes, stems, and soil on a weekly basis in a simulated vineyard housed in a greenhouse setting. The study concluded that there were no significant differences between the modified and unmodified strain when assessed for fitness, survival, and impacts to the ecological balance of the microflora.⁷⁹

In another experiment, *S. cerevisiae* was genetically modified to express the human coagulation Factor XIIIa (rhFXIIIa). No difference in survival rates between the modified and unmodified was noted under natural soil/water suspension, soil/medium suspension, and wastewater conditions.⁸⁰

In a laboratory setting, *S. cerevisiae* can mate with *S. paradoxus* with relative ease. In the natural environment, even though the two species coexist, *S. cerevisiae* demonstrates an own-species

⁷⁹ Bauer, F. F., Dequin, S., Pretorius, I. S., Shoeman, H., Wolfaardt, G., Schroeder, M. B., & Grossmann, M. K. (2004). The assessment of the environmental impact of genetically modified wine yeast strain. *Bulletin de l'OIV-Office International de la Vigne et du Vin*, 77(881-882), 515-528.

⁸⁰ Fujimura, H., Sakuma, Y., & Amann, E. (1994). Survival of genetically-engineered and wild-type strain of the yeast *Saccharomyces cerevisiae* under simulated environmental conditions: A contribution on risk assessment. *Journal of Applied Bacteriology*, 77(6), 689–693.

[illegible]

⁸¹ Greig, D. (2009). Reproductive isolation in *Saccharomyces*. *Heredity*, 102(1), 39–44.

⁸³ Ando A., Suzuki C., Shima J. (2005). Survival of genetically modified and self-cloned strain of commercial Baker's yeast in simulated natural environments: environmental risk assessment. *Applied and Environmental Microbiology*. 71(11). 7075-7082.

cerevisiae strain notified in this MCAN is not expected to have increased survivability compared to other strains in the environment. [REDACTED]
[REDACTED]

[REDACTED]
[REDACTED] As reported by Reuter *et al.*⁸⁴ insects play a role in dispersion. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED] it is not expected that the modified strain, [REDACTED] would be dispersed through insect vectors to a degree greater than well-known *S. cerevisiae* strains commonly found in nature.

Generally, the published literature shows no significant differences in the survivability of modified *S. cerevisiae* compared to the unmodified parental strain under certain conditions. Therefore, it is reasonable to conclude that the modified *S. cerevisiae* strain notified in this MCAN is not expected to have increased survivability compared to other strains in the environment.

As reported by Sniegowski *et al.* 2002, a condition necessary for growth of modified or unmodified yeast is a nutrient rich environment.⁸⁵ From Sniegowski *et al.* it would be reasonable to conclude that *S. cerevisiae* can survive in the environment, such as in fluxes or soil, and when enough nutrients are present, can grow through cell budding. It is likewise expected that the

⁸⁴ Reuter, M., Bell, G., & Greig, D. (2007). Increased outbreeding in yeast in response to dispersal by an insect vector, Supplemental Data. *Current Biology*, 17(3), R81–R83.

⁸⁵ Sniegowski P.D., Dombrowski P.G., Fingerman E. (2002). *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* coexist in a natural woodland site in North America and display different levels of reproductive isolation from European conspecifics. *FEMS Yeast Research*. 1. 299-306.

modified strain would be comparable in their ability to survive in fluxes and soils of broad-leaved trees and can grow if a sufficient nutrient supply was available.

Bauer *et al.*⁸⁶ reports the results of greenhouse trials evaluating the release and viability of modified *S. cerevisiae*. The conditions in the greenhouse were designed to simulate a vineyard. Yeast populations were applied by spraying to grapes, leaves, stem and soil weekly for one year and the progress of the modified yeast was evaluated against blocks of plantings left untouched. The authors report that:

although a high concentration of yeast was sprayed, few S. cerevisiae strains could be isolated at any given time. The yeast population in the sprayed blocks was otherwise very similar to the one found on the control vines, indicating that the commercial or GM yeast did not affect the overall ecological balance of the micro-flora. Furthermore, no significant differences between the behavior of the genetically modified and the parental strain could be detected.

In year two, the same pattern was observed, with no significant difference with regard to presence in the greenhouse vineyard or cell numbers, suggesting that “the GM yeasts did not benefit from any specific advantage in terms of overall fitness when released in the vineyard.”

Fujimura *et al.*⁸⁷ studied a genetically engineered strain of *S. cerevisiae* employed for the industrial production of the human coagulation Factor XIIIa (rhFXIIIa) in a survival study under simulated environmental conditions. The strain was introduced into natural soil/water suspension, into soil/medium suspension, and into wastewater. The homologous strain devoid of the recombinant plasmid and the homologous strain bearing the 2 micron-based vector plasmid without the rhFXIIIa-encoding DNA insert were compared. After intervals, samples of cell

⁸⁶ Bauer, F. F., Dequin, S., Pretorius, I. S., Schoeman, H., Wolfaardt, G., Schroeder, M. B., & Grossman, M. K. (2004). The assessment of the environmental impact of genetically modified wine yeast strain. *Bulletin de l'O.I.V.*, 77 (881-882), 515–528.

⁸⁷ Fujimura, H., Sakuma, Y., & Amann, E. (1994). Survival of genetically-engineered and wild-type strain of the yeast *Saccharomyces cerevisiae* under simulated environmental conditions: A contribution on risk assessment. *Journal of Applied Bacteriology*, 77(6), 689–693.

suspensions were taken and viable cell numbers were determined by plating on antibiotic-containing medium. No differences in survival rates could be detected for the plasmid-bearing and plasmid-less strain under the three environmental conditions tested (soil/water suspension, YEPD medium/soil, and wastewater), suggesting that the presence of plasmid does not confer selective advantages on the survival of the yeast cells. The authors conclude that, even after accidental release of the engineered yeast cells into the environment, elimination rates would be comparable to those for non-recombinant yeast strain. The study noted that excessive growth of fungi and bacteria may be a condition that inhibits the survival of yeast cells (p. 691) in soil. Soil and wastewater were noted as poor in nutrients for the growth of yeast cells as well (p. 693).

The papers we reviewed discussed survival under pH, temperature and nutrient conditions that are well within the parameters normally associated with yeast survival. The modified strain are not designed to be tolerant to conditions outside these normal pH, temperatures, salinity, and nutrient conditions.

Based on the absence of demonstrated conditional differences between wild type and the MCAN strain, it is reasonable to conclude that the modified strain is not expected to behave differently from *S. cerevisiae* strains commonly found in nature.

3.4 Anticipated Biological Interactions with Target Organisms and Other Organisms

- Host range: The modified *S. cerevisiae* strain is not anticipated to require a host range for survival, similar to wild type *S. cerevisiae*.
- Target organism: The modified *S. cerevisiae* strain is not anticipated to act upon any target organisms, similar to the unmodified strain.
- Competitors: Fermentation conditions such as temperature and nutrients are often favorable for other microorganisms, such as gram-positive bacteria, which compete with the yeast for nutrients and produced compounds toxic to the yeast such as lactic and acetic acids. The modified *S. cerevisiae* is not anticipated to out-compete other microorganisms. [REDACTED]
[REDACTED]
[REDACTED]

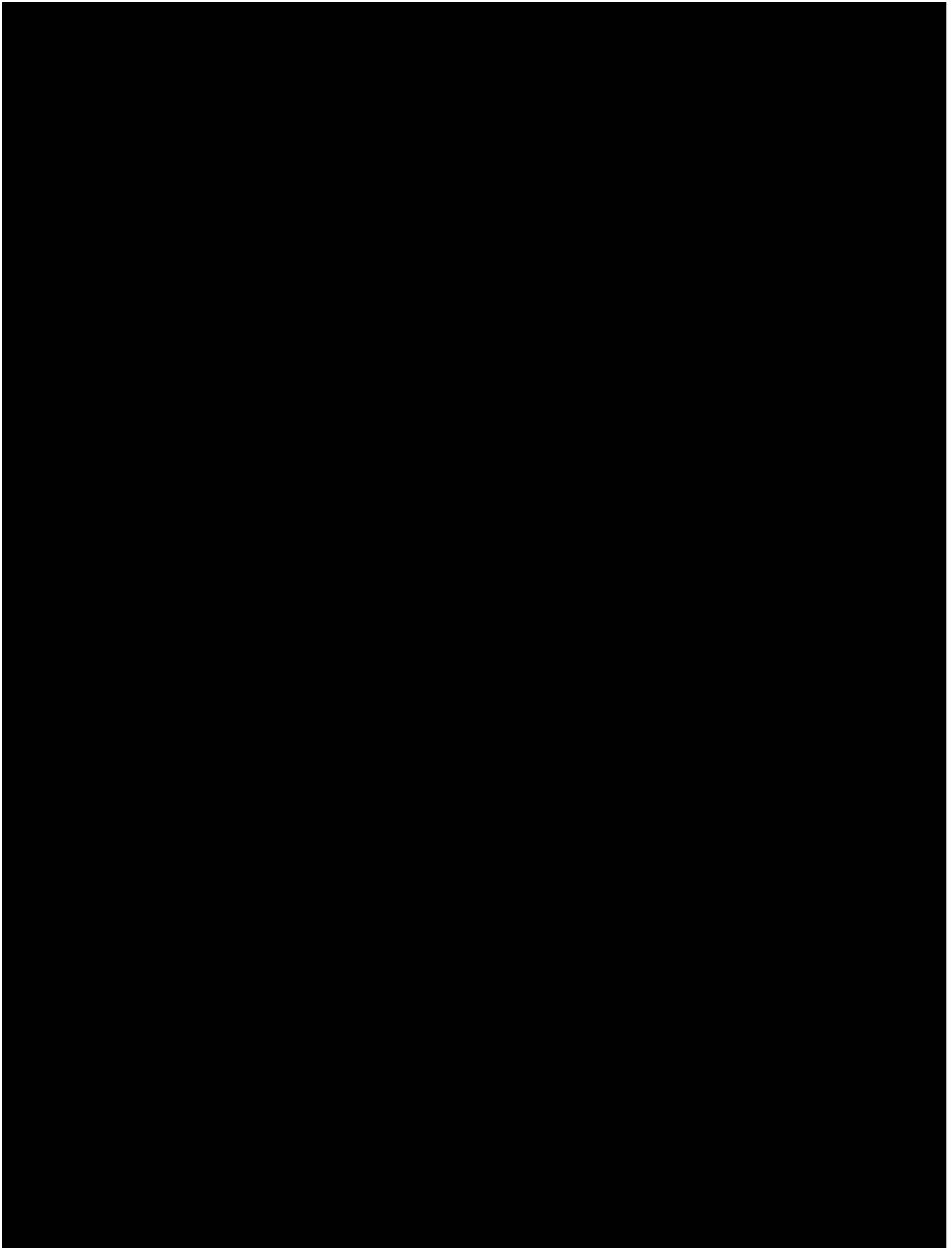
- Prey: The modified *S. cerevisiae* strain is not anticipated to prey upon other organisms, similar to the unmodified strain.
- Hosts: The MCAN microorganism is not designed to be a host or to infect or feed upon another living organism. The production strain is not of the type that exhibits parasitic behavior with grapevine (*Vitis vinifera* L.) plants. The parasitic behavior of certain strain is considered novel and associated only with certain *S. cerevisiae* that exhibit filamentous forms.
- Symbionts: The production strain is not designed for a symbiotic relationship, no symbiont beneficiary is anticipated.
- Parasites: No significant interactions with parasites are reported in the literature based on the search that was conducted for this submission.
- Pathogens: The MCAN strain is not designed to enhance any pathogen such as *Escherichia coli* or *Clostridium botulinum*.

3.5 Pathogenicity, Infectivity, Toxicity, Virulence

3.5.1 Nonhuman Pathogenicity

3.5.1.1 Donor Organisms

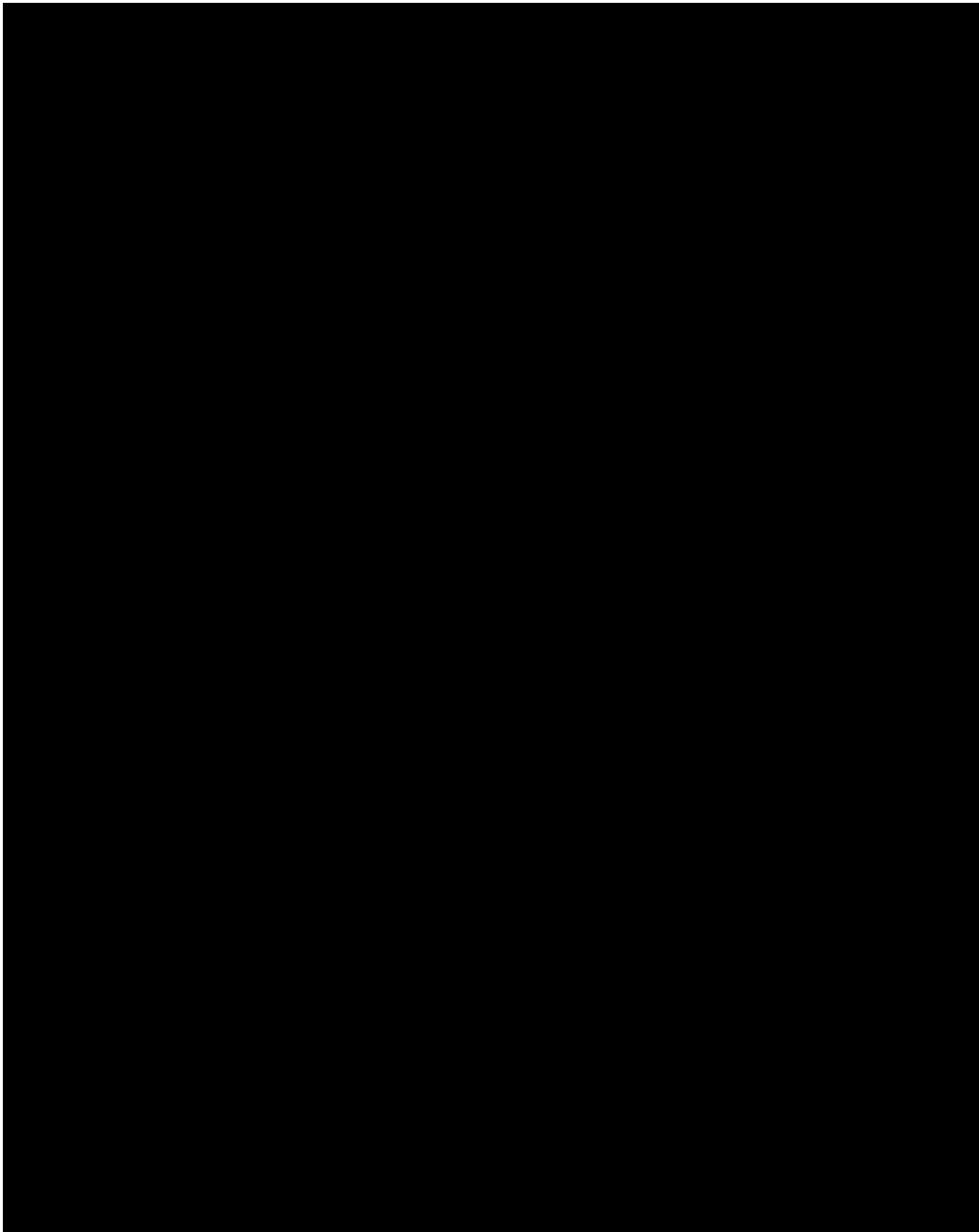
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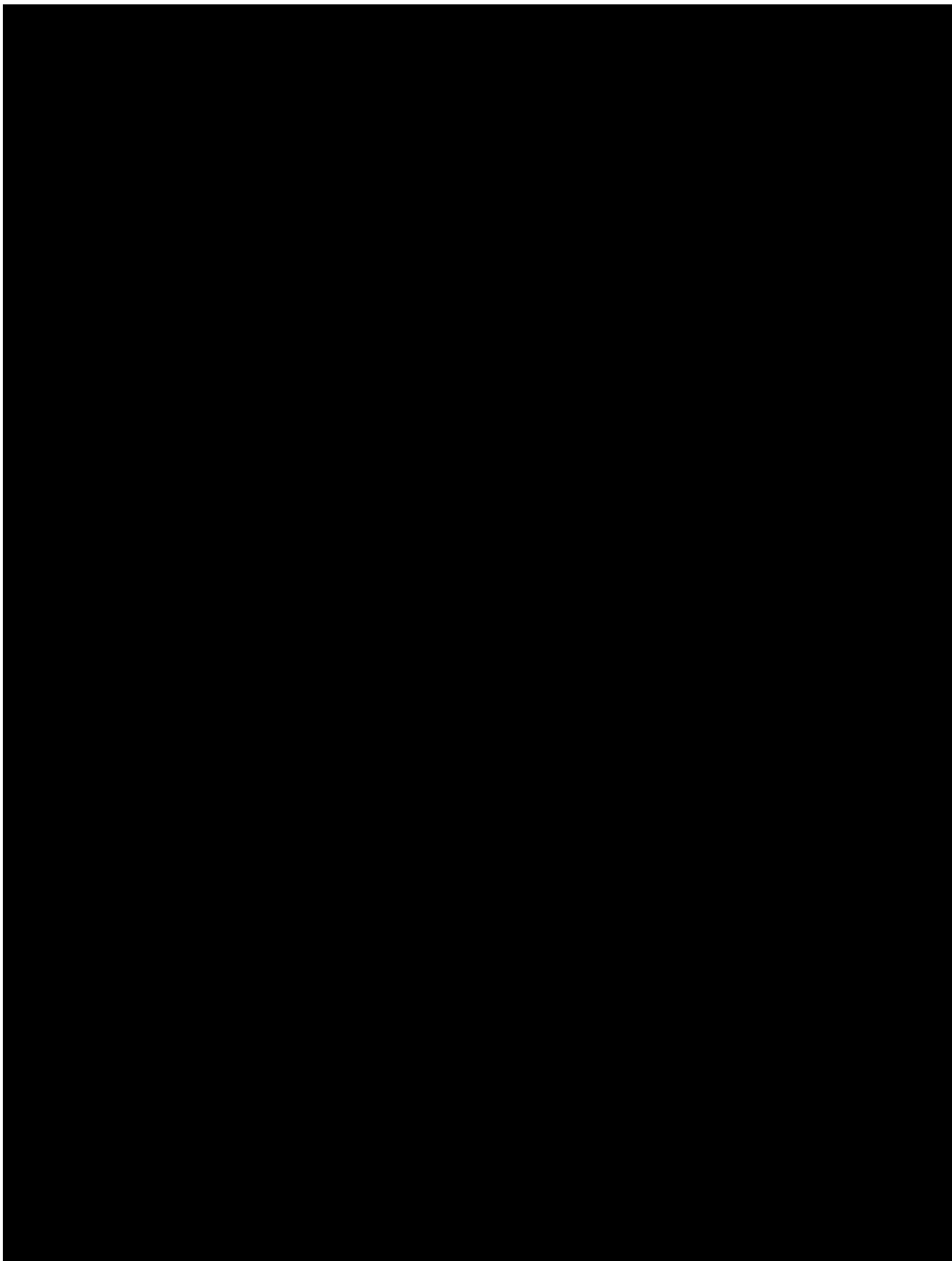
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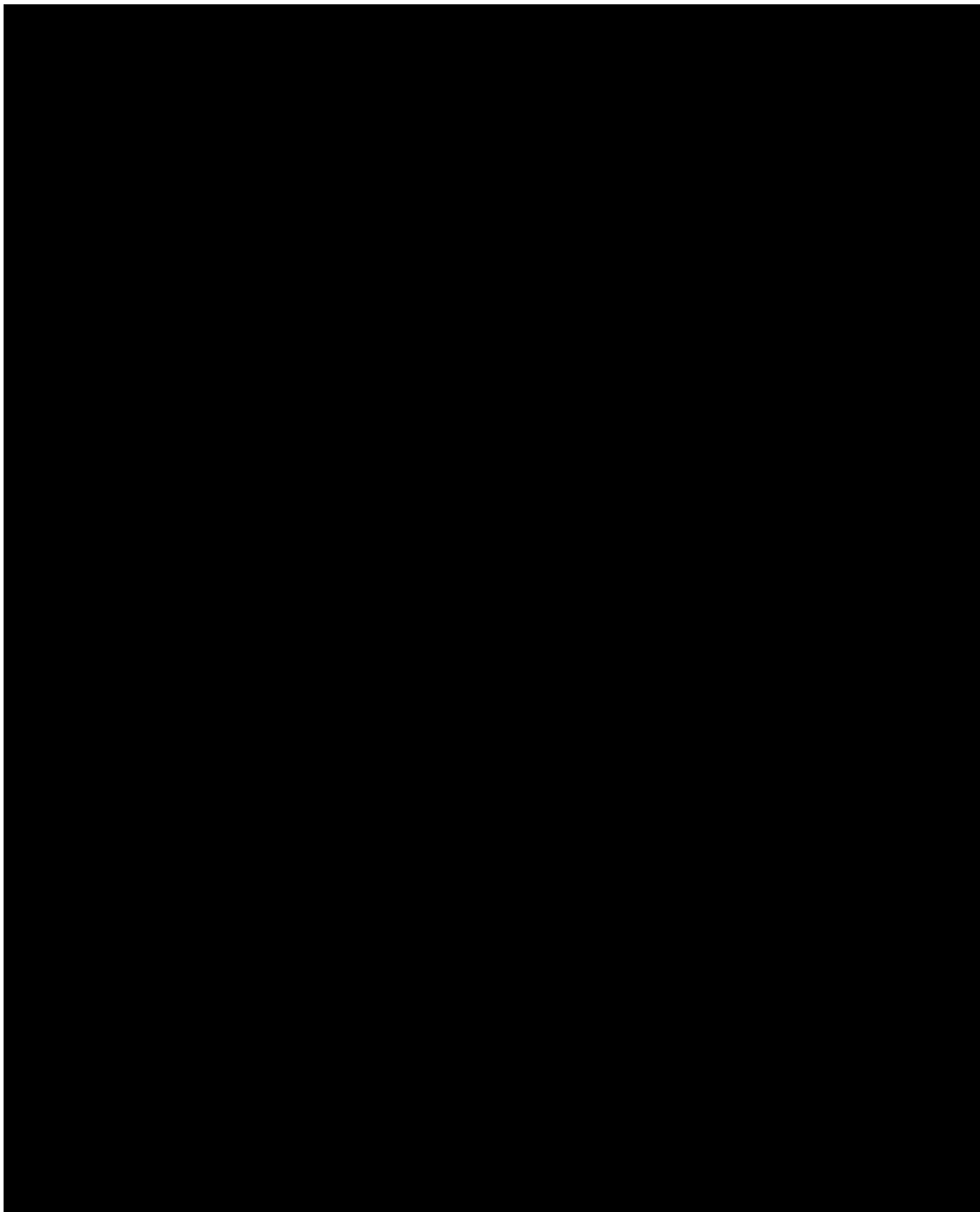
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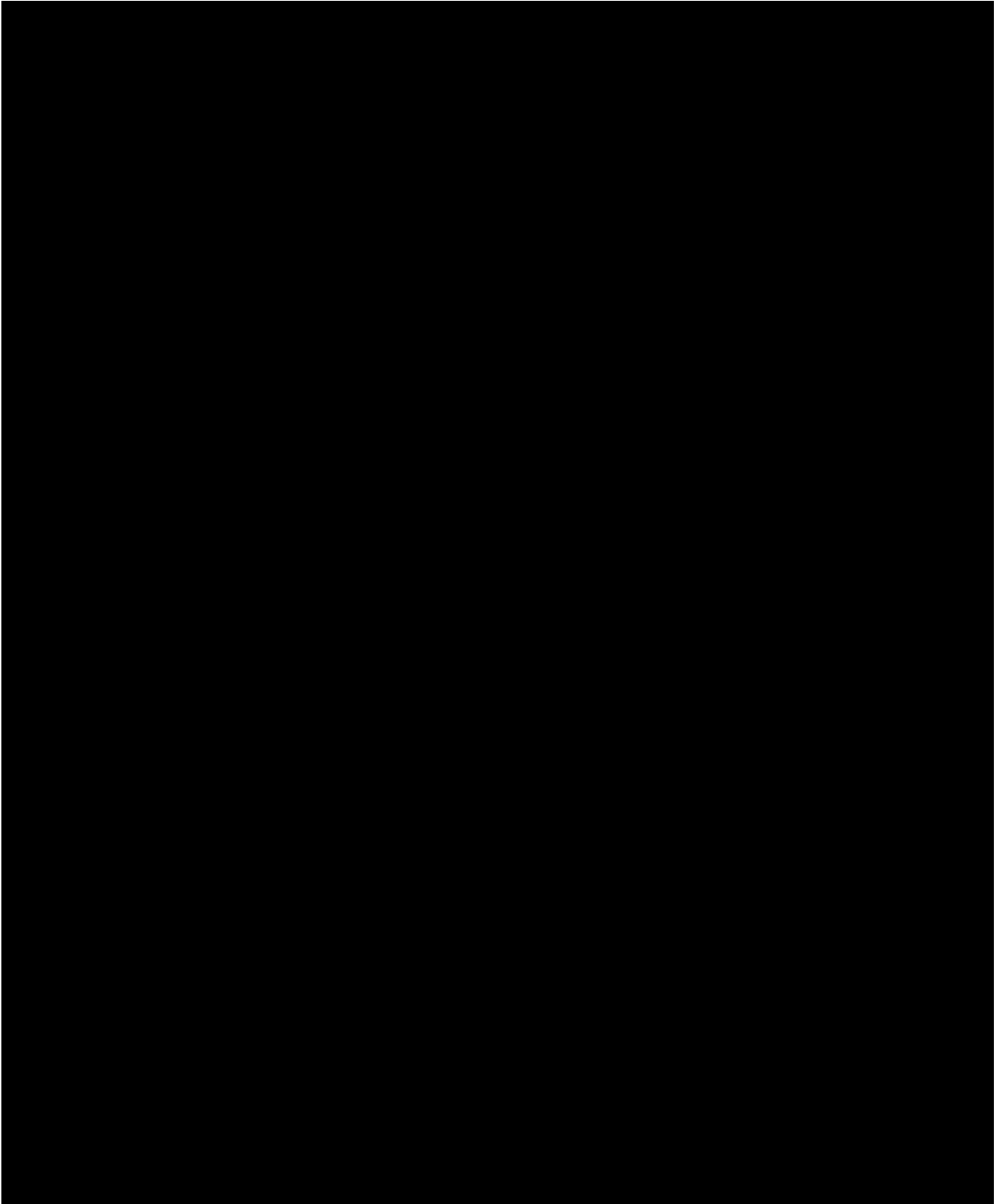
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3.5.1.2 Host Organism

Published studies characterize *S. cerevisiae* as strictly an obligate or opportunistic pathogen. The literature is generally supportive of the view that the potential is rare for *S. cerevisiae* to be a source of non-human species infectivity in healthy animals or to produce toxins.

Because the modifications to *S. cerevisiae* were not shown through a literature search to be toxic or yield different toxicological results from the unmodified strain, surrogate information on the recipient strain is offered for the purpose of evaluating the anticipated behavior of the production strain.

Regarding non-human pathogenicity, the Environment Canada Risk Assessment Summary Conducted Pursuant to the New Substances Notification Regulations (Organisms) (NSNR[o]) of the Canadian Environmental Protection Act, 1999, EAU-288: *S. cerevisiae* strain ECMo01 (August 23, 2006) concluded that “reports of *S. cerevisiae* pathogenicity to insects, birds, fish, animals, and plants in the available scientific literature are exceedingly rare.” This risk assessment noted a reported case associating *S. cerevisiae* with chronic diarrhea in a dog.¹¹² In EPA’s Final Risk Assessment of *Saccharomyces cerevisiae*¹¹³ the Agency discussed the ability of a fungus to impair the host’s immune capabilities in connection the anticipated effect on non-human species and concluded that *S. cerevisiae* is nonpathogenic. EPA states (p. 4):

The cell walls of most fungi have the capacity to impede the immune response of the host. In a study to determine the overall pathogenicity of a number of yeasts used in industrial processes, animals exposed to both high levels of S. cerevisiae and cortisone demonstrated a greater ability of the fungus to colonize compared with those animals treated with only the yeast. However, the animals suffered no ill-effects from exposure to S. cerevisiae (Holzschu et al., 1979). Therefore, this study suggests that even with the

¹¹² Milner R.J., Picard J, Tustin R. (1997). Chronic episodic diarrhoea associated with apparent intestinal colonisation by the yeasts *Saccharomyces cerevisiae* and *Candida famata* in a German shepherd dog: case report. *Journal of the South African Veterinary Association*. 68(3): 147-149.

¹¹³ U.S. EPA. (1997). *Saccharomyces cerevisiae* Final Risk Assessment: Attachment I--Final Risk Assessment of *Escherichia Coli* K-12 Derivatives. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Biotechnology Program under the Toxic Substances Control Act (TSCA).

addition of high levels of an immunosuppressant agent, S. cerevisiae appears to be nonpathogenic.

The Agency goes on to conclude (p. 9) that “The organism is not a plant or animal pathogen. Despite the fact that *S. cerevisiae* is ubiquitous in nature, it has not been found to be associated with disease conditions in plants or animals.”

Certain strain of *S. cerevisiae*, especially strain isolated from fermenting Champagne wine must, can slow down growth or cause necrosis in young grapevine plantlets in a laboratory setting.¹¹⁴ This study demonstrated that a general yeast strain from the America Type Culture Collection showed little effect on the growth of young grapevine plantlets compared to yeast strain isolated from Champagne wine must, and did not provide data on the ability of the yeast to act as a pathogen in the wild or on adult grapevine plants.

EPA’s Final Risk Assessment identifies the potential for *S. cerevisiae* to be pathogenic toward other yeast. As EPA states on p. 3:

There have been no reports of isolates of S. cerevisiae that produce toxins against either humans or animals. However, S. cerevisiae has been shown to produce toxins against other yeasts. These toxins, termed “killer toxins”, are proteins or glycoproteins produced by a range of yeasts. The yeasts have been genetically modified to alter activity and are used in industrial settings as a means of controlling contamination of fermentation systems by other yeasts (Sid et al., 1988)

EPA further observes that:

. . the species does carry linear, double-stranded plasmids, which can be transmitted to other Saccharomyces. These plasmids carry genes that encode the “killer toxins” discussed above [sic] can be transferred from one Saccharomyces to another. Therefore,

¹¹⁴ Gognies S, Belarbi A, Barka EA, 2001. *Saccharomyces cerevisiae*, a potential pathogen towards grapevine, *Vitis vinifera*. *FEMS Microbiology Ecology*. 37:143-150].

gene constructs involving the incorporation of traits using these linear plasmids should be considered to be nonstable.

A public literature search was performed to determine the ability of *S. cerevisiae* to cause adverse health effects in non-human species. A study on diseased prawns under aquaculture conditions noted about 0.8% of the yeast infections were due to *S. cerevisiae* with the majority of the yeast infections (98.4%) due to *Metschnikovia bicuspidate*. The LD₅₀ for *S. cerevisiae* when healthy prawns were infected was determined to be 2.0×10^3 CFU/prawn. It is worth noting that the yeast strain used for the LD₅₀ studies were strain isolated from diseased prawns and may have been a pathogenic strain. The authors recognized that the aquaculture cultivation conditions of the prawns may have contributed to the ability of the yeast to infect the prawns.¹¹⁵

A laboratory *S. cerevisiae* feeding study in *Caenorhabditis elegans* showed that infection and death could be caused by progressive distension and accumulation of *S. cerevisiae* in the intestinal lumen of the *C. elegans*, which led to the reversible production of reactive oxygen species. No yeast exposure levels were reported and the report only indicates that the yeast were diluted to 20 mg/liter prior to exposure. Although observed under controlled laboratory conditions, there were no findings on whether these symptoms were found in *C. elegans* in the wild and no published reports were located to indicate that *C. elegans* in the wild had these symptoms.¹¹⁶

A probiotic study in which rainbow trout (*Oncorhynchus mykiss*) fry were fed up to 10% of their diet with viable *S. cerevisiae* for a four week period had no adverse effects observed in development, growth, or mortality.¹¹⁷

¹¹⁵ Chen S.C., Chen Y.C., Kwang J. Manopo I., Wang P.C., Chaung H.C., Liaw L.L., Chiu S.H. *Metschnikowia bicuspidate* dominates in Taiwanese cold-weather yeast infections of *Macrobrachium rosenbergii*. *Dis Aquat Organ*. 2007 May 9;75(3). 191-9.

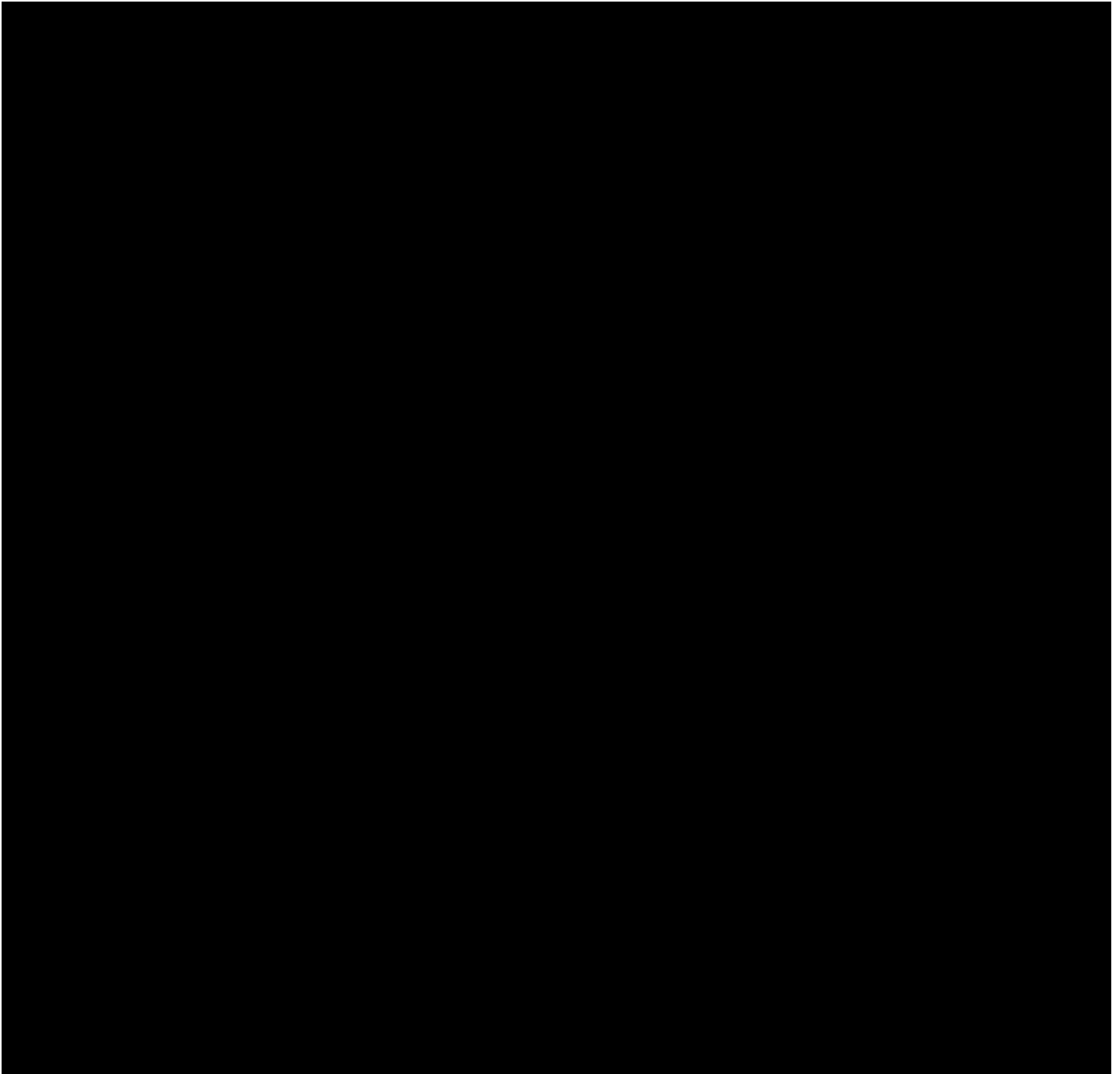
¹¹⁶ Jain C., Yun M., Politz S.M., Rao R.P. (2009). A pathogenesis assay using *Saccharomyces cerevisiae* and *Caenorhabditis elegans* reveals novel roles for yeast AP-1, Yap1, and host dual oxidase BLI-3 in fungal pathogenesis. *Eukaryot Cell*. 8(8). 1218-27.

¹¹⁷ Pooramini, M.; Kamali, A.; Hajimoradloo, A.; Alizadeh, M.; Ghorbani, R. (2009). Effect of using yeast (*Saccharomyces cerevisiae*) as probiotic on growth parameters, survival and carcass quality in rainbow trout *Oncorhynchus mykiss* fry. *International Aquatic Research* 2009. 1(1). 39-44.

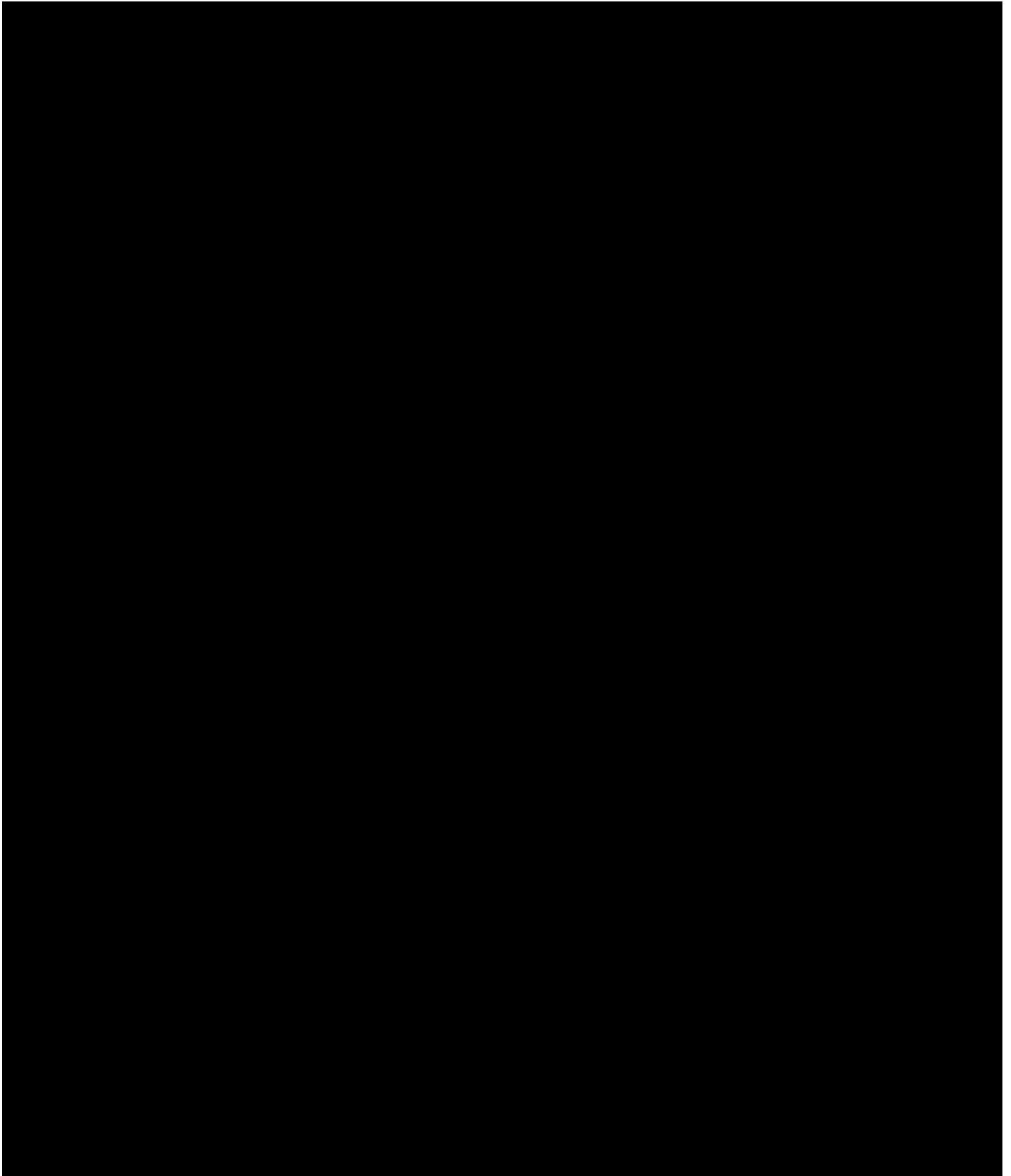
In conclusion, *S. cerevisiae* can act as an opportunistic pathogen under artificial aquaculture or laboratory conditions for prawns and *C. elegans*, respectively, and a feeding study in rainbow trout showed no detectable adverse effects. In non-human species, *S. cerevisiae* is no more than an opportunistic pathogen.

3.5.2 Effects in Humans

3.5.2.1 Donor Organisms



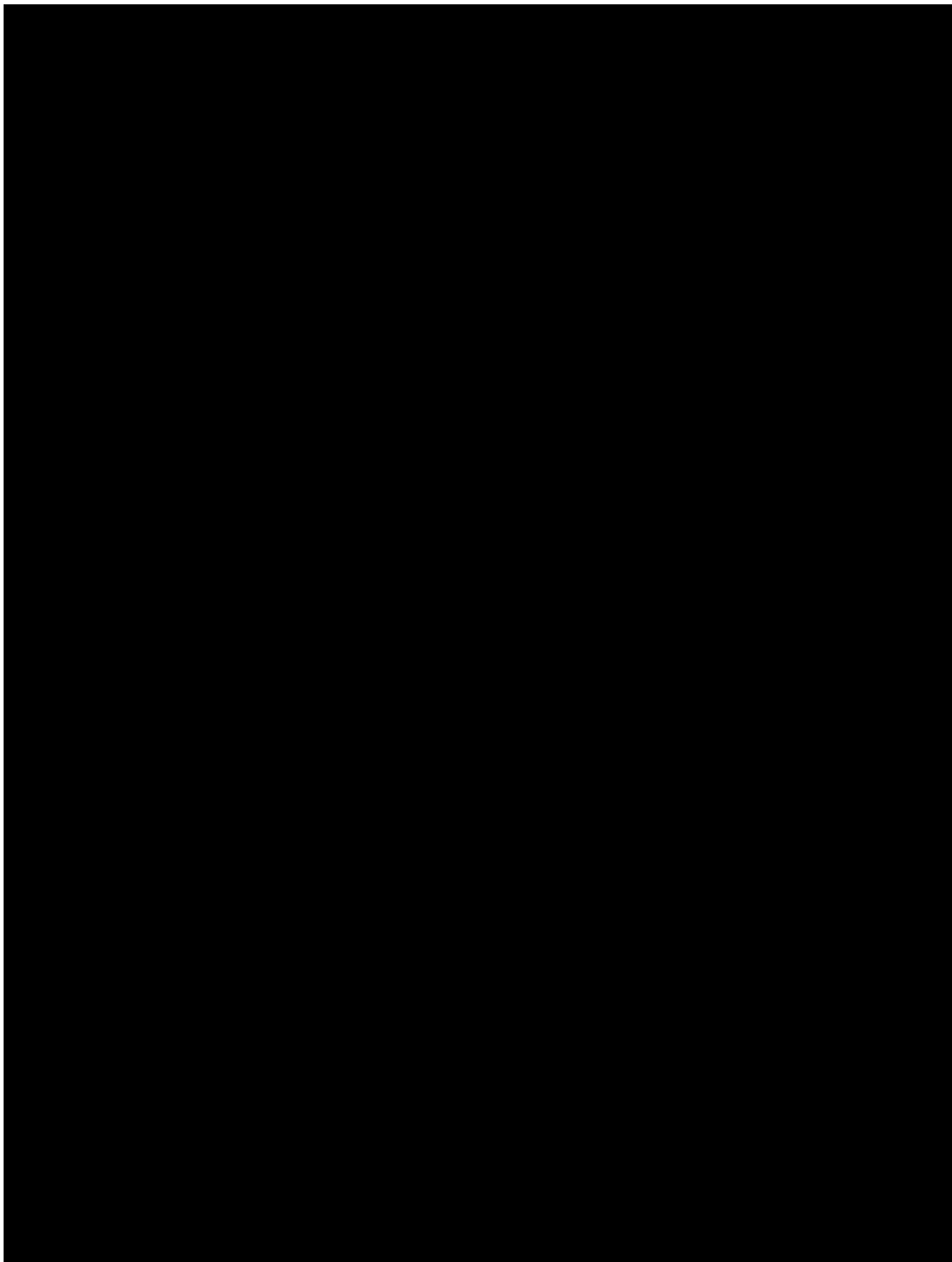
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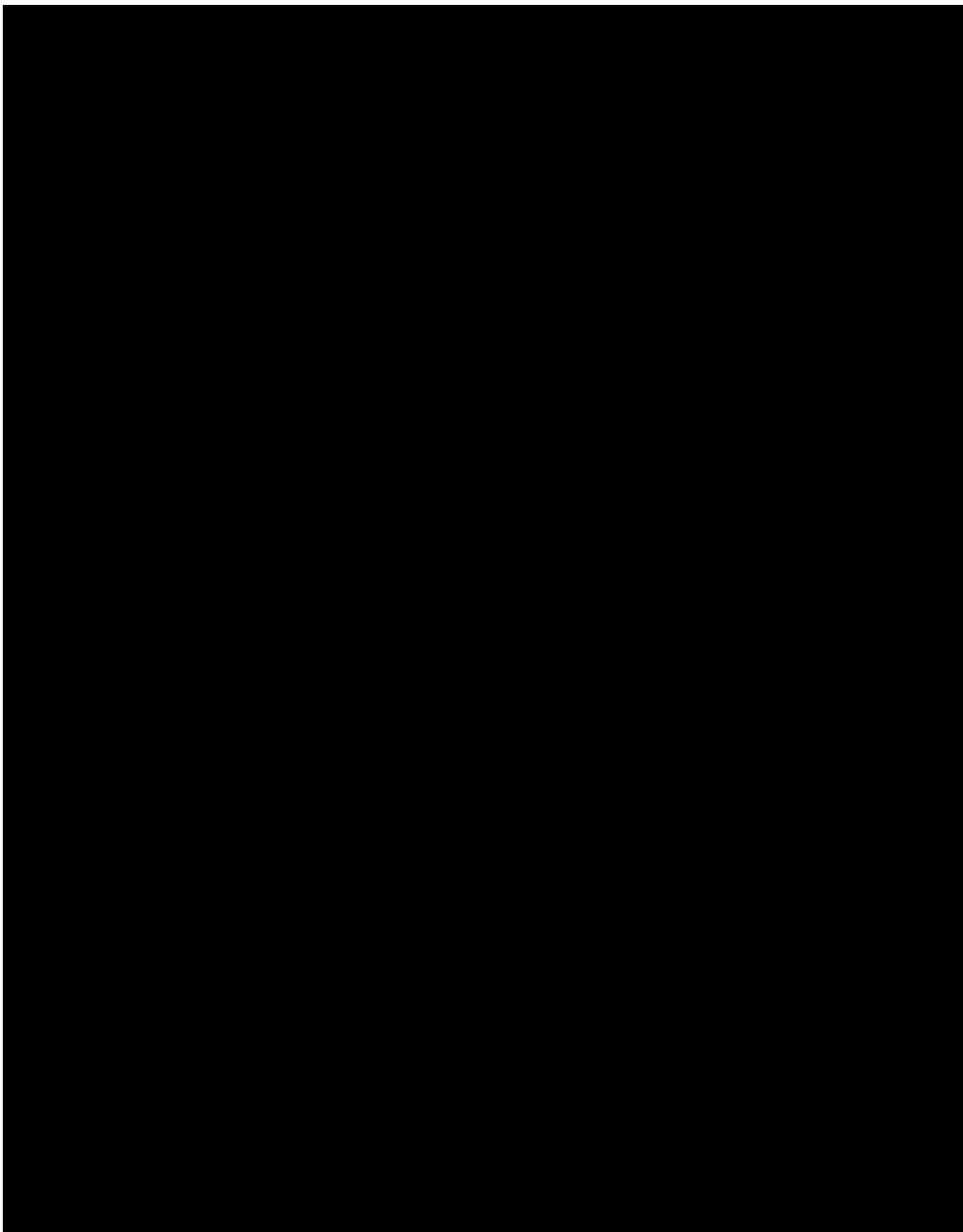
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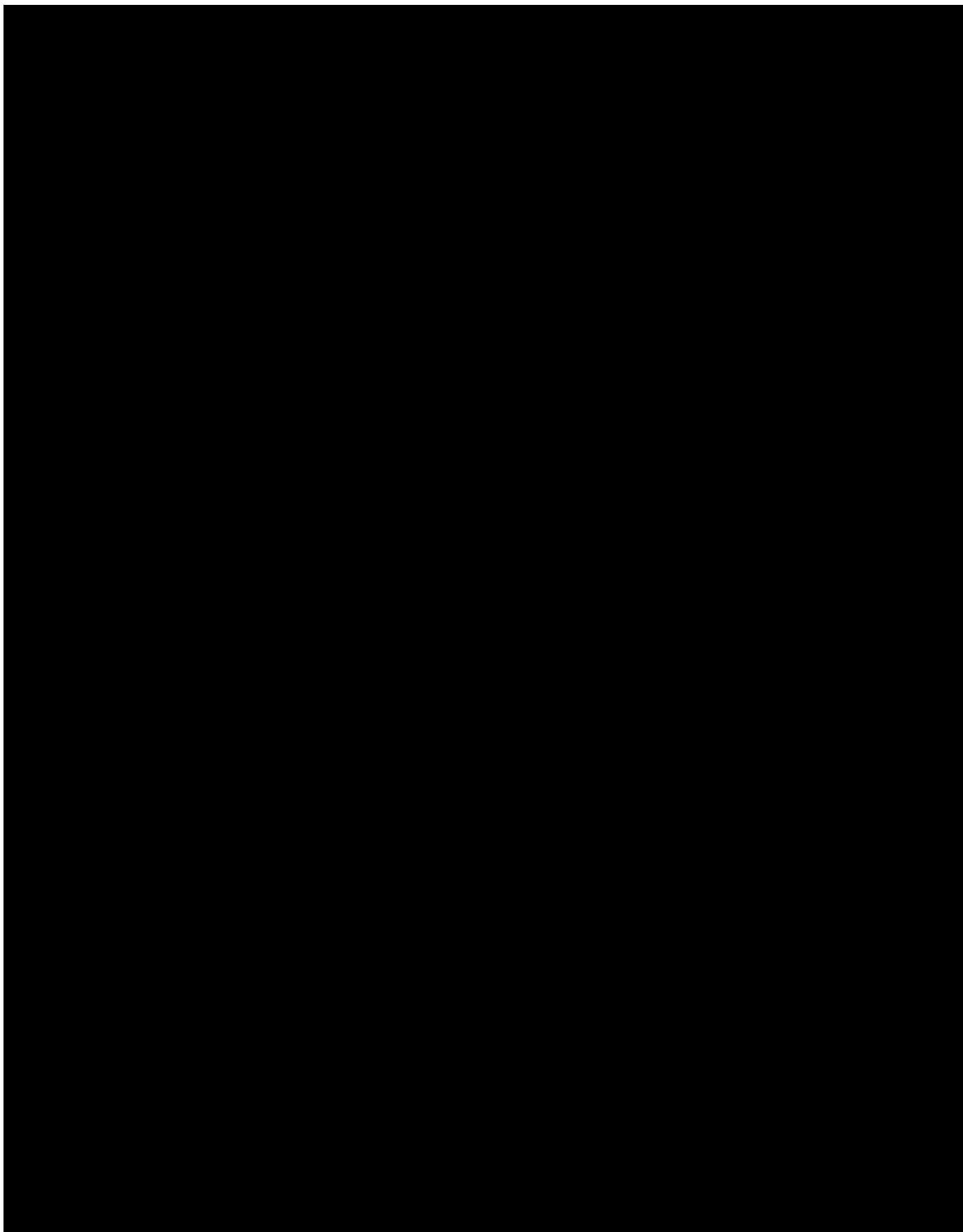
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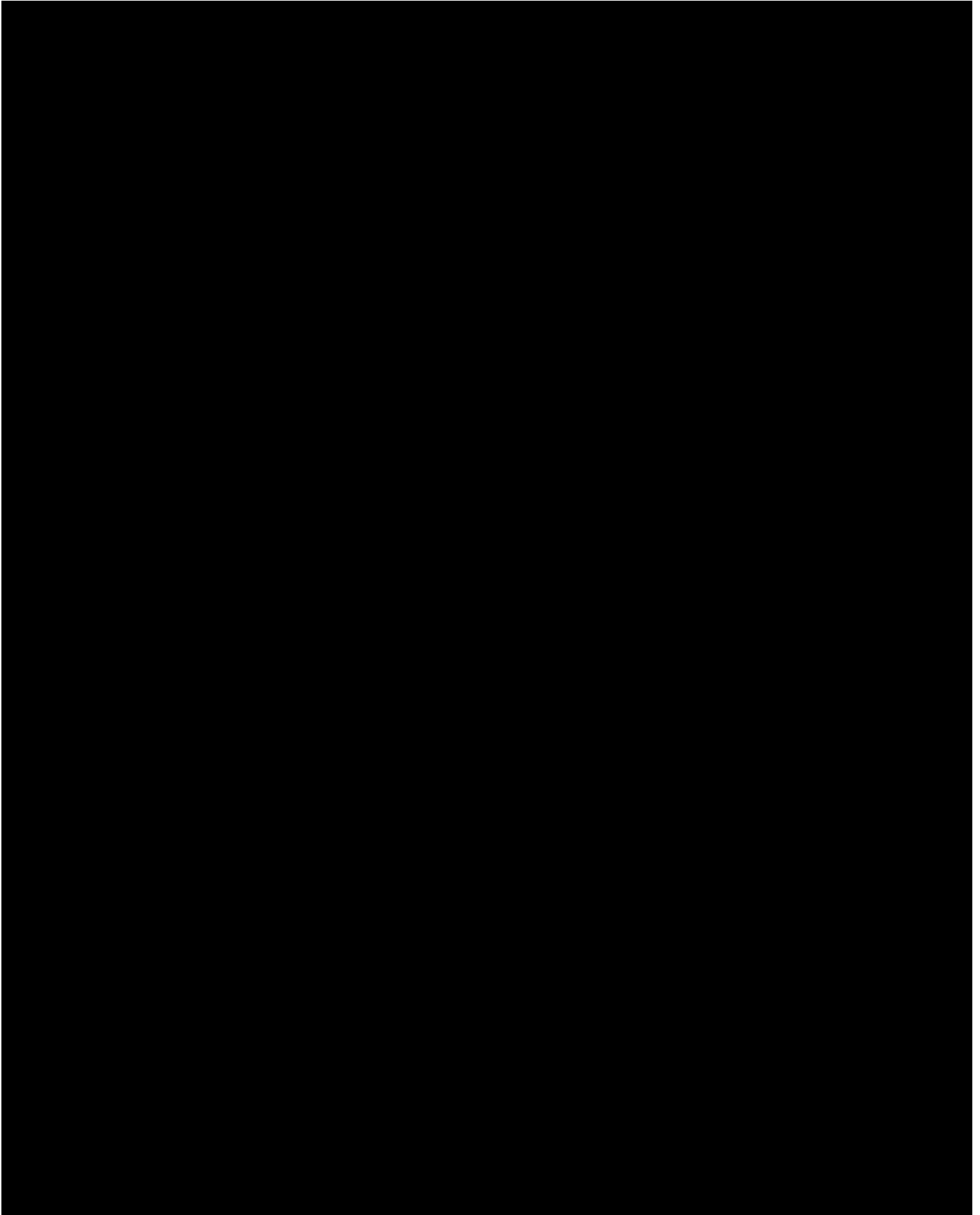
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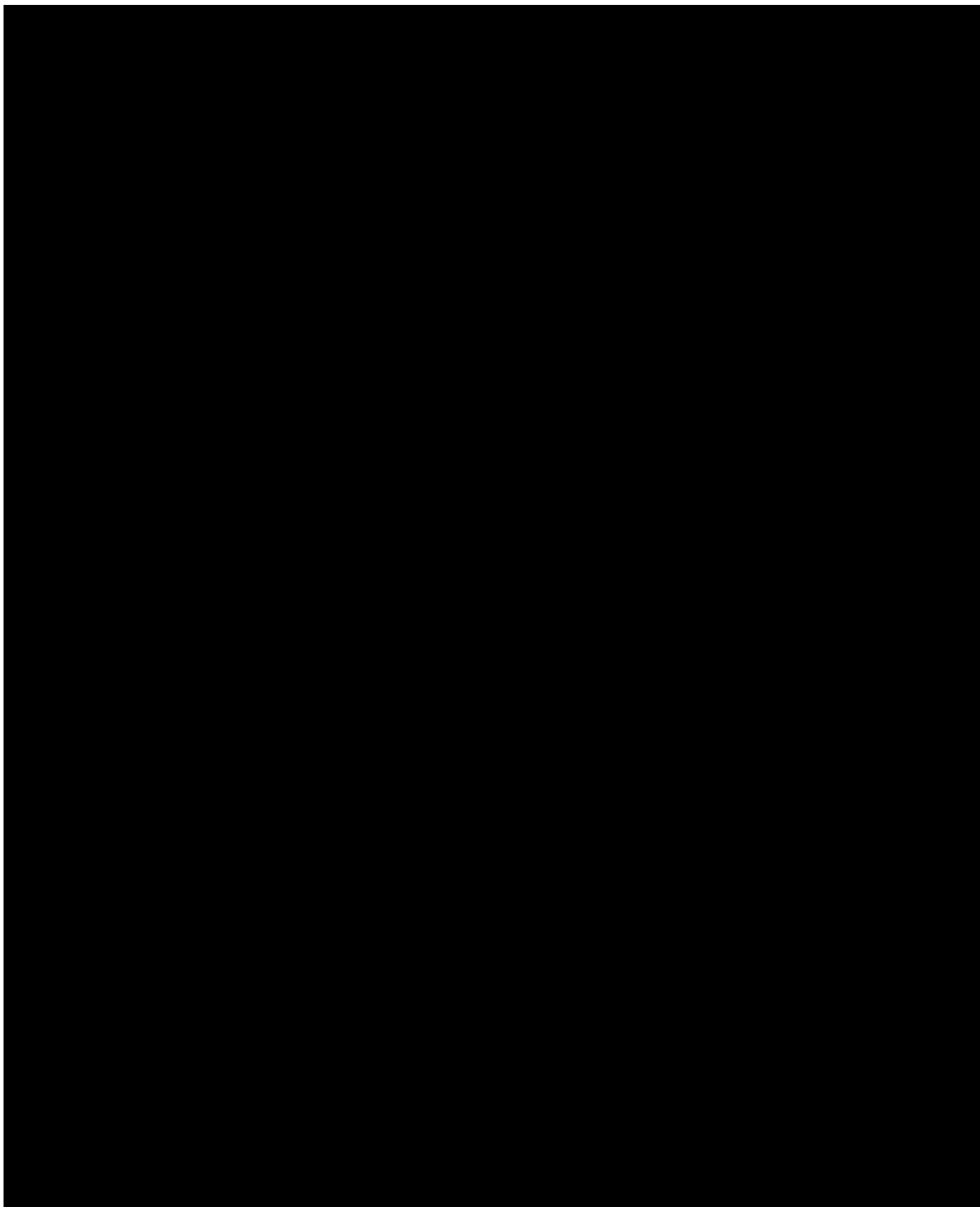
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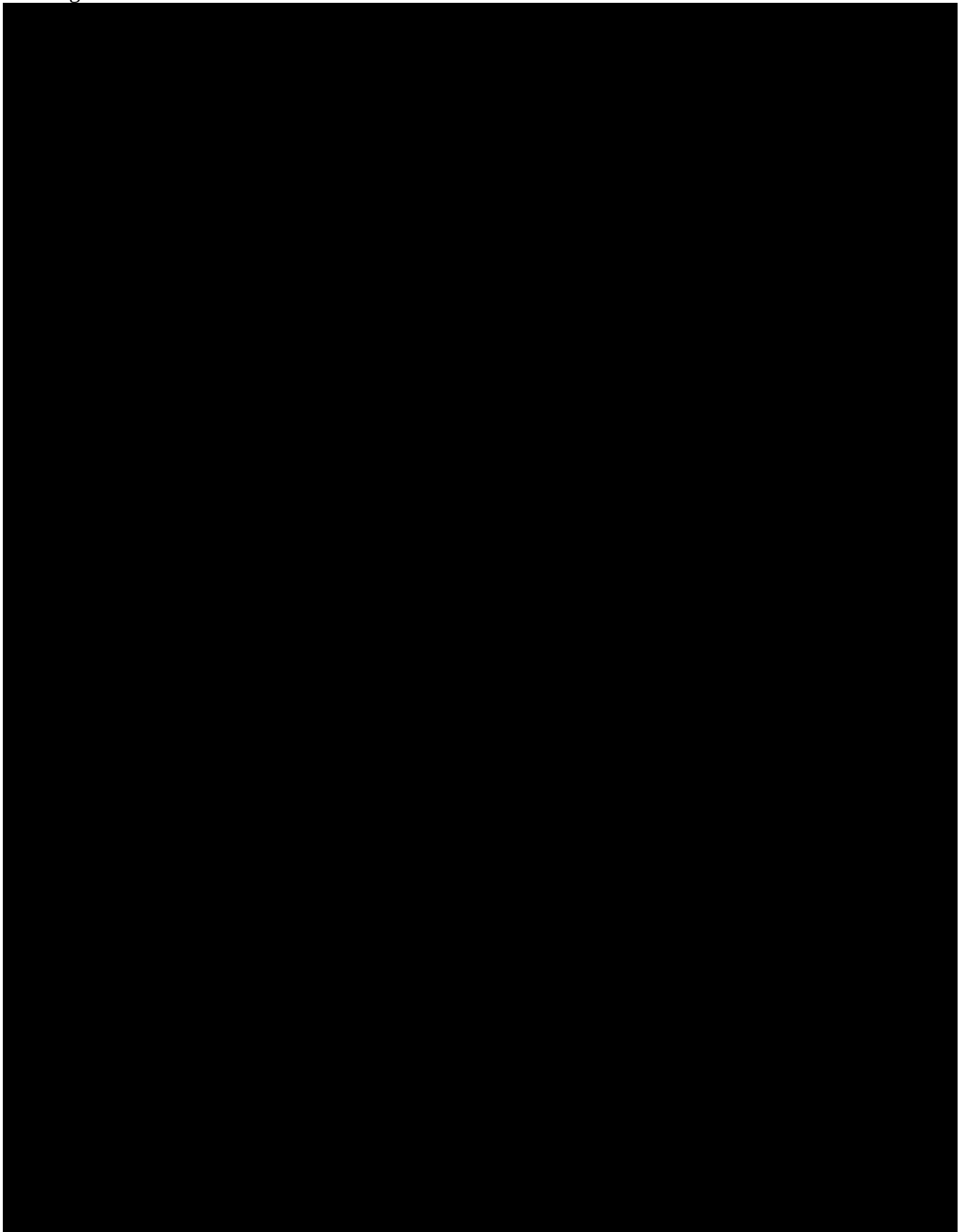
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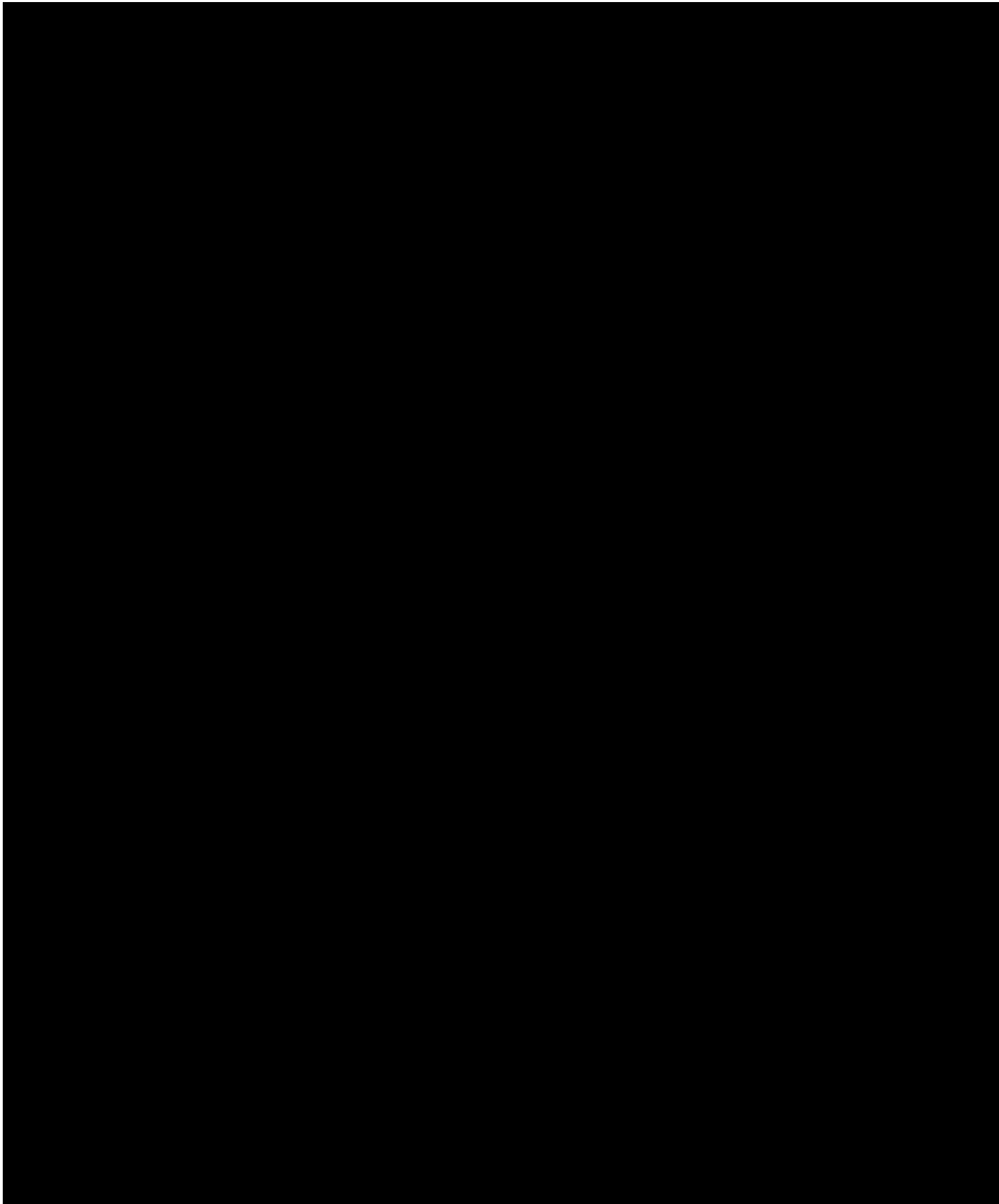
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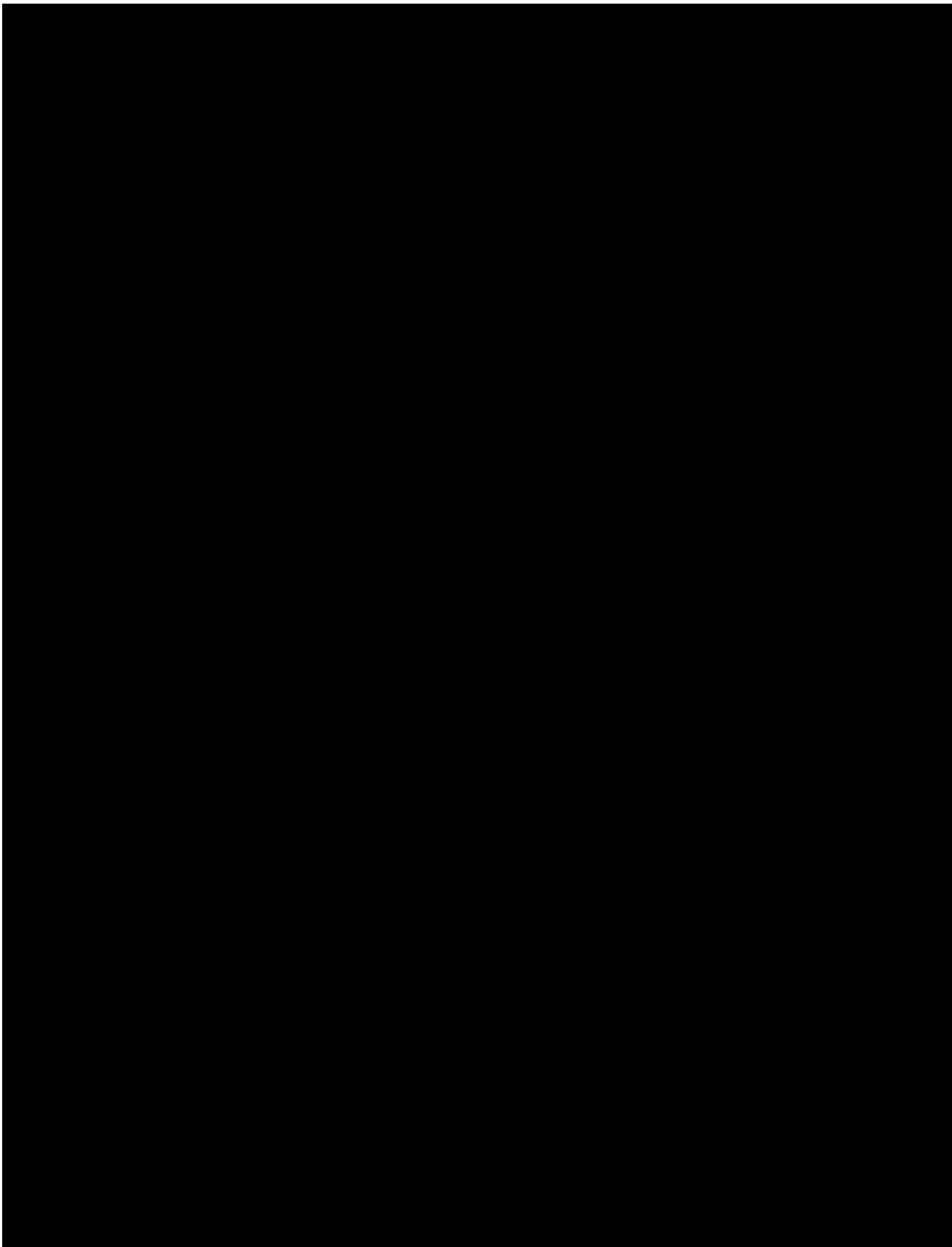
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3.5.2.2 Host Organism

Because the modifications to the host organism was not shown through a literature search to be toxic or yield different toxicological results from the wild-type strain, surrogate information on the recipient strain is offered for evaluating the anticipated behavior of the production strain.

ATCC classifies *S. cerevisiae* as a BSL-1 organism based upon the fact that the organism is not known to cause disease in healthy humans. A review of the U.S. Centers for Disease Control (CDC) website did not yield any involvement of *S. cerevisiae* in adverse health effects. A search using “*Saccharomyces cerevisiae*” and pathogen* did not turn up any studies that indicated that the strain contains pathogenic genes. The literature reports that *S. cerevisiae* is an opportunistic

pathogen. A 2006 chapter by McCusker¹⁷⁰ provides a list of *S. cerevisiae* infections described in the literature. The list includes infections in patients with AIDS; it does not identify which of the other patients were otherwise immunocompromised. A 2005 report by Muñoz *et al.* described three (3) ICU patients that had *S. cerevisiae* fungemia at Hospital General Universitario.¹⁷¹ As part of the report, the authors searched MEDLINE for reports of *S. cerevisiae* fungemia since 1966. Their search returned only fifty-seven additional reported cases. Since *S. cerevisiae* is commonly used in the biotechnology industry, Murphy and Kavanagh examined the potential pathogenicity of *S. cerevisiae*.¹⁷² They concluded that *S. cerevisiae* can be regarded as an opportunistic pathogen for the immunocompromised, but one of low virulence.

As EPA recognized in its Final Risk Assessment of *Saccharomyces cerevisiae* (February 1997; p. 9), “[m]any scientists believe that under appropriate conditions any microorganism could serve as an opportunistic pathogen.” The Agency concluded that *S. cerevisiae* has an extensive history in food processing and neither it nor other closely related species “has been associated with pathogenicity toward humans or has been shown to have adverse effects on the environment” (p.2). Specifically, with respect to human exposure, EPA concluded on p. 3 of the Final Risk Assessment that:

There are individuals who may ingest large quantities of S. cerevisiae every day, for example, people who take the yeast as part of a “health food” regimen. Therefore, studies were conducted to ascertain whether the ingestion of large numbers of these yeasts might result in either colonization, or colonization and secondary spread to other organs of the body. It was found that the installation of very large numbers of S. cerevisiae into the colons of animals would result in both colonization and passage of the yeasts to draining lymph nodes. It required up to 10¹⁰ S. cerevisiae in a single oral treatment to rats to achieve a detectable passage from the intestine to the lymph nodes

¹⁷⁰ McCusker, J. H. (2006). *Saccharomyces cerevisiae*: An Emerging and Model Pathogenic Fungus. In *Molecular Principles of Fungal Pathogenesis* (pp. 245–259). ASM Press.

¹⁷¹ Muñoz P, Bouza E, Cuenca-Estrella M, Eiros JM, Pérez MJ, Sánchez-Somolinos M, Rincón C, Hortal J, Peláez T. (2005). *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. *Clinical Infectious Disease*. 40: 1625-1634.

¹⁷² Murphy A, Kavanah K. (1999). Emergence of *Saccharomyces cerevisiae* as a human pathogen: implications for biotechnology. *Enzyme and Microbial Technology*. 25:551-557.

(Wolochow et al., 1961). The concentrations of S. cerevisiae required were well beyond those that would be encountered through normal human daily exposure.

EPA concluded that: “*Saccharomyces*, as a genus, present low risk to human health or the environment. Criteria used to differentiate between species are based on their ability to utilize specific carbohydrates without relevance to pathogenicity. Nonetheless, this risk assessment applies to those organisms that fall under the classical definition of *S. cerevisiae* as described by van der Walt (1971).” The production strain falls under the classical definition described by van der Walt (1971). For the foregoing reasons, it is respectfully submitted that the MCAN strain is nonpathogenic to humans.

3.5.3 Virulence

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[REDACTED]
[REDACTED]
[REDACTED]
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[REDACTED] As described in EPA’s *S. cerevisiae* Final Risk Assessment, the principal virulence factors associated with yeast are phospholipase A and lysophospholipase.¹⁷³ EPA observes in its Final Risk Assessment (p. 4) that:

A number of individual virulence factors have been identified as being associated with the ability of yeasts to cause disease. The principal virulence factors associated with yeasts appear to be phospholipase A and lysophospholipase. It is believed that these enzymes enhance the ability of the yeast to adhere to the cell-wall surface and result in colonization as a first step in the infectious process. Nonpathogenic yeast had

¹⁷³ U.S. EPA. (1997). *Saccharomyces cerevisiae* Final Risk Assessment: Attachment I--Final Risk Assessment of *Escherichia Coli* K-12 Derivatives. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Biotechnology Program under the Toxic Substances Control Act (TSCA).

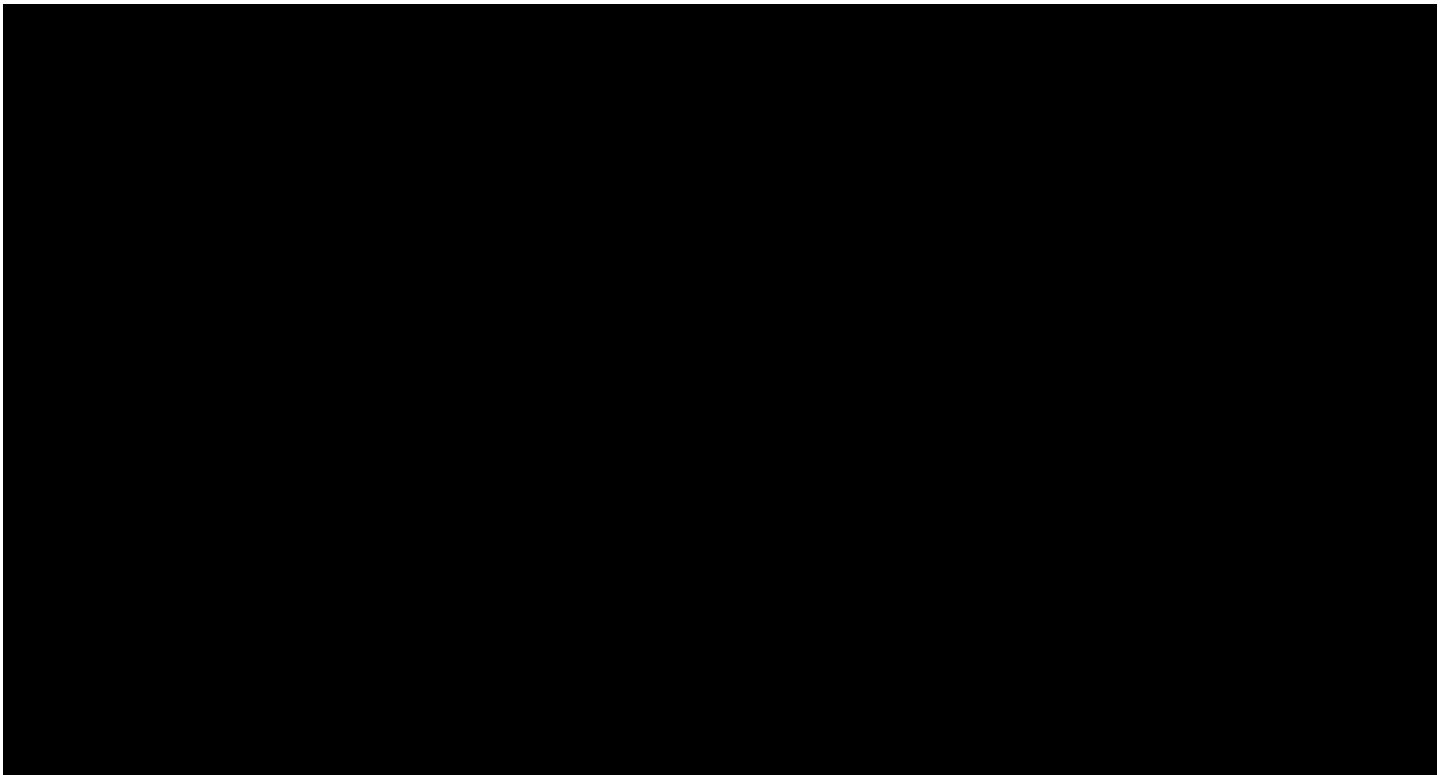
considerably lower phospholipase activities. Of a wide range of fungi assayed for phospholipase production, S. cerevisiae was found to have the lowest level of activity (Barrett-Bee et al., 1985). Therefore, based on the phospholipase virulence factor S. cerevisiae is considered a nonpathogenic yeast.

EPA has concluded that *S. cerevisiae* has low potential to exhibit phospholipase virulence and is nonpathogenic to humans. The agency concluded in this regard on p. 4 that “*S. cerevisiae* does not carry virulence factors to humans or animals.” The MCAN strain is not expected to have a level of activity different from the wild type strain and should be considered likewise nonpathogenic.

Based on these results, the strain is considered nonpathogenic as the genetic modifications are metabolic genes and have not been identified to be linked with pathogenicity or virulence.

3.5.4 Immunologic Reactions

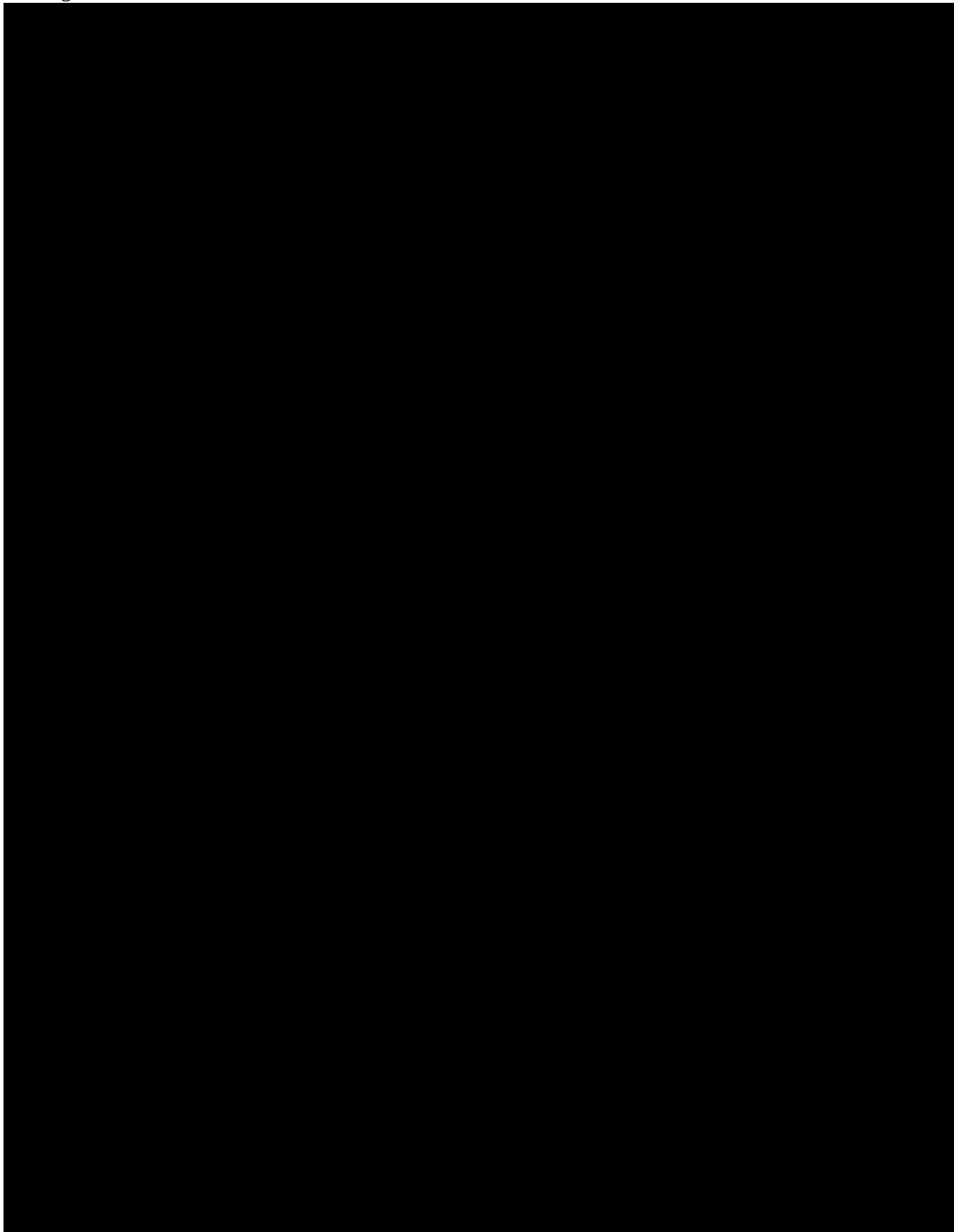
3.5.4.1 Information on Immunologic Potential



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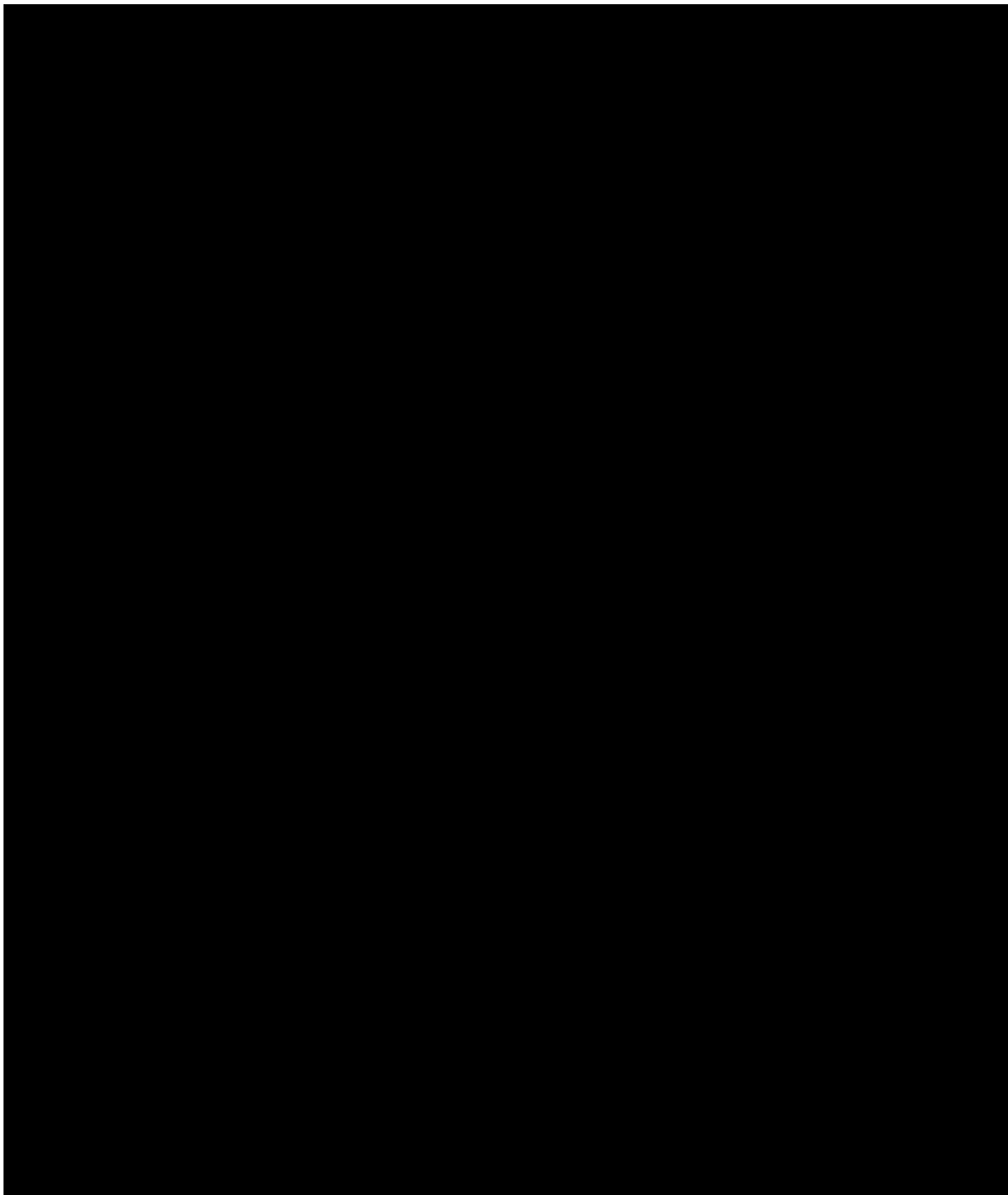
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3.5.4.2 Published Studies

Yeast is a ubiquitous part of human environments, an ingredient in everyday diet, and is commonly used in the biotechnology industry. The literature reports that yeast allergies, particularly *Saccharomyces cerevisiae*, are very rare and there have been a limited number of isolated reported cases. Of these isolated cases, a majority were inhalation allergies.^{179, 180} In a few cases, ingestion may cause allergies to humans.^{181, 182, 183} Our literature search on the safety of *Saccharomyces cerevisiae* revealed that cases of allergies of yeast are extremely uncommon, rarely reported, and the conclusions ambiguous. In the case studies we reviewed, it is important to note the overall sensitivities of the patients, in which no single study indicates a sole

¹⁷⁹ Pajno, G., Passalacqua, G., Salpietro, C., Vita, D., Caminiti, L., & Barberio, G. (2005). Looking for immunotolerance: A case of allergy to baker's yeast (*Saccharomyces cerevisiae*). *European Annals of Allergy and Clinical Immunology*, 37(7), 271—272.

¹⁸⁰ Baldo, B. A., & Baker, R. S. (1988). Inhalant Allergies to Fungi: Reactions to Bakers' Yeast (*Saccharomyces cerevisiae*) and Identification of Bakers' Yeast Enolase as an Important Allergen. *International Archives of Allergy and Immunology*, 86(2), 201–208.

¹⁸¹ Bansal, R. A., Tadros, S., & Bansal, A. S. (2017). Beer, Cider, and Wine Allergy. *Case Reports in Immunology*, 2017, 1–4.

¹⁸² Pajno, G., Passalacqua, G., Salpietro, C., Vita, D., Caminiti, L., & Barberio, G. (2005). Looking for immunotolerance: A case of allergy to baker's yeast (*Saccharomyces cerevisiae*). *European Annals of Allergy and Clinical Immunology*, 37(7), 271—272.

¹⁸³ Airola, K., Petman, L., & Mäkinen-Kiljunen, S. (2006). Clustered sensitivity to fungi: Anaphylactic reactions caused by ingestive allergy to yeasts. *Annals of Allergy, Asthma & Immunology*, 97(3), 294–297.

sensitivity to *Saccharomyces cerevisiae* but are rather associated with hypersensitive patients often with additional autoimmune symptoms and/or general fungal allergies. Our conclusion is that the modified *S. cerevisiae* strain does not raise safety concerns.

Baldo and Baker examined the results of skin prick tests and radioallergosorbent tests (RASTs) and found positive reactions to protein extracts from *S. cerevisiae* and purified enolase from *S. cerevisiae* in people with inhalant allergies to airborne fungi.¹⁸⁴ The study emphasized that although the results demonstrate a high incidence of positive skin tests and RAST reactions in those subjects, it does not mean that if the subjects were exposed to the proteins, an allergic response would occur. While these tests demonstrate that the subjects have antibodies against the proteins, the presence of an antibody does not equate to an allergic response.

A more recent study by Horner *et al.* examined the ability of commercially produced fungal enzyme extracts on IgE antibody reactivity by RAST, including *S. cerevisiae* enzymes.¹⁸⁵ The paper did not examine the sensitivity of subjects to the fungal enzymes, supporting the conclusion that commercially produced enzyme extracts could be used as source material for clinical allergen testing.

No further studies examining worker exposure and allergy responses in the baking and ethanol industry were found. Therefore, exposure to the modified *S. cerevisiae* is not expected to elicit any allergic response to workers during ethanol production.

3.5.5 Antibiotic Resistance (ABR)

¹⁸⁴ Baldo, B. A., & Baker, R. S. (1988). Inhalant Allergies to Fungi: Reactions to Bakers' Yeast (*Saccharomyces cerevisiae*) and Identification of Bakers' Yeast Enolase as an Important Allergen. *International Archives of Allergy and Immunology*, 86(2), 201–208.

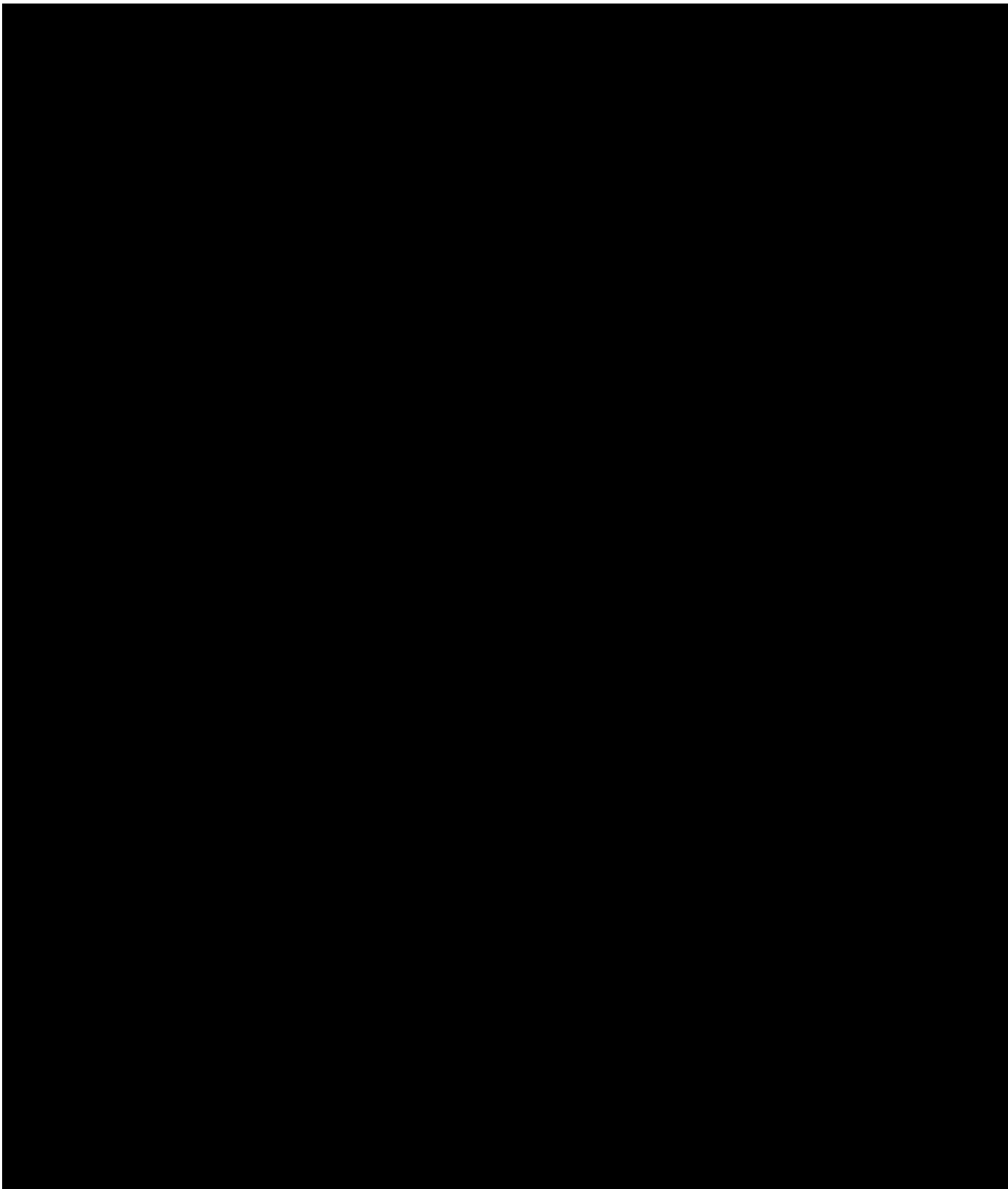
¹⁸⁵ Horner, W. E., Armstrong, M., El-Dahr, J., McCants, M., Reese, G., Kobernick, A. K., & Lehrer, S. B. (2008). Prevalence of IgE reactivities in mold-allergic subjects to commercially available fungal enzymes. *Allergy and Asthma Proceedings*, 29(6), 629–635.

Based on the absence of demonstrated adverse effects for the parental strain and for the inserted intergeneric sequences, it is reasonable to conclude that the modified strain is not expected to be resistant to antibiotics, or more tolerant to metals, antifungals, or pesticides.

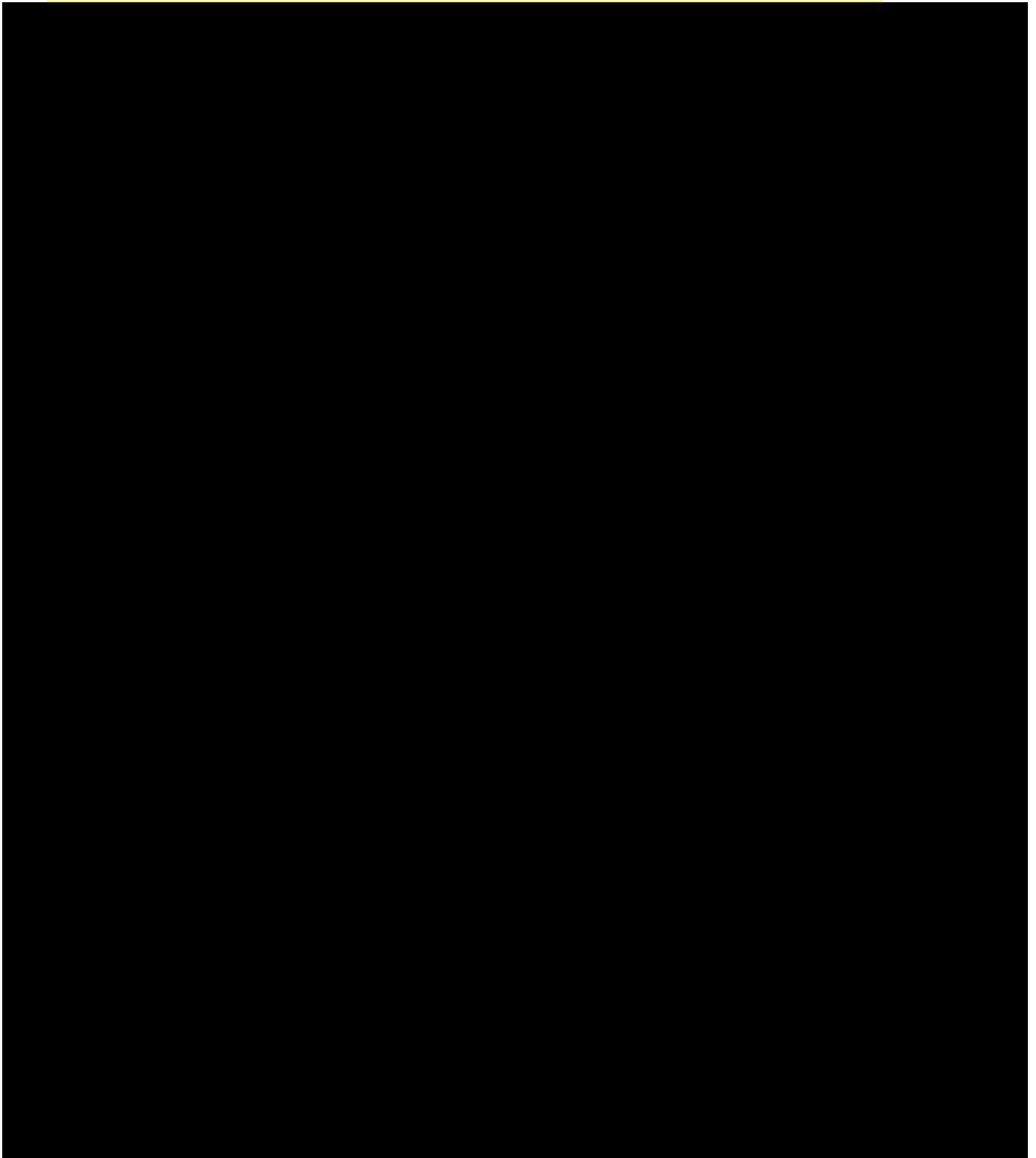
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3.5.7 Capacity for Genetic Transfer under Laboratory and Environmental Conditions



3.5.8 Anticipated Involvement in Biogeochemical or Biological Cycling Processes

A search of the scientific literature using the search terms “*Saccharomyces cerevisiae*” and nutrient cycle terms such as “carbon cycle,” “nitrogen cycle,” “phosphorus cycle,” and “sulfur cycle” demonstrates that *S. cerevisiae* is not known to play a lead role in these processes. One article that is relevant describes the metabolism of sulfur into the sulfur amino acids in *S. cerevisiae*. The review pertains to the biological sulfur cycle, which consists of: (1) degradation; (2) dissimilatory oxidation; (3) dissimilatory reduction; and (4) assimilatory reduction. Yeast and

all eukaryotic plants and microorganism carry out assimilatory reduction to metabolize sulfur.¹⁹¹ The authors state that *S. cerevisiae* does not abnormally influence this cycle. Based on the absence of demonstrated adverse effects for the [REDACTED] strain and for the inserted intergeneric sequences, it is reasonable to conclude that the modified strain is not expected to have an adverse effect on biological or biogeochemical cycles.

3.5.9 Summary of Safety Assessment

The MCAN strain is “well-characterized” and meet the criteria of 40 C.F.R. § 725.421. As EPA noted in its 1997 Final Risk Assessment for *S. cerevisiae*¹⁹² (p. 12), because the recipient microorganism was found by the Agency to have little potential for adverse effects, “introduced genetic material meeting the specified criteria” of § 725.421 “would not likely significantly increase potential for adverse effects.” [REDACTED]

U.S. EPA. (1997). *Saccharomyces cerevisiae* Final Risk Assessment: Attachment I--Final Risk Assessment of *Escherichia Coli* K-12 Derivatives. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Biotechnology Program under the Toxic Substances Control Act (TSCA).

4. Byproducts during Manufacture, Processing, Use and Disposal of the Strain

4.1 Byproducts of Yeast Production Facilities

4.2 Byproducts from Ethanol Production Facilities

The major byproducts of fuel ethanol production are carbon dioxide, process water and process solids. Carbon dioxide is vented to the atmosphere or may be recovered as a purified product. The process water can be recirculated and reused back into the ethanol process. The process solids, which consists mostly of inactivated biomass and residual proteins and grain fiber are recovered and sold as distillers' products for animal feed. Minor byproducts of fuel ethanol production include: plant oils, glycerol, lactic acid and acetic acid.

5. Importation Volume, Manufacture Volume, and Transportation

6. Site Controlled by the Submitter

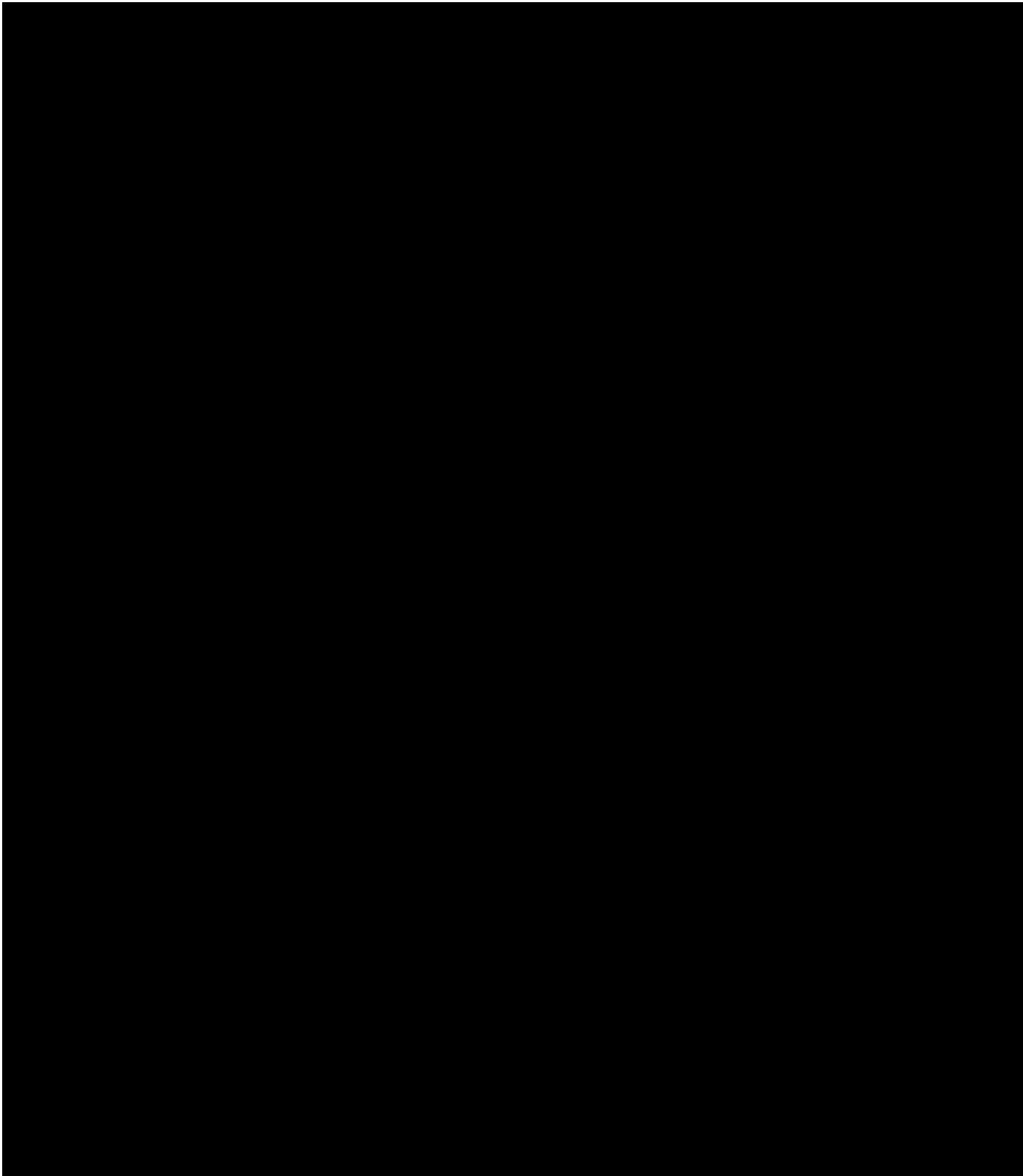
6.1 Manufacture of

6.1.2 Raw Materials

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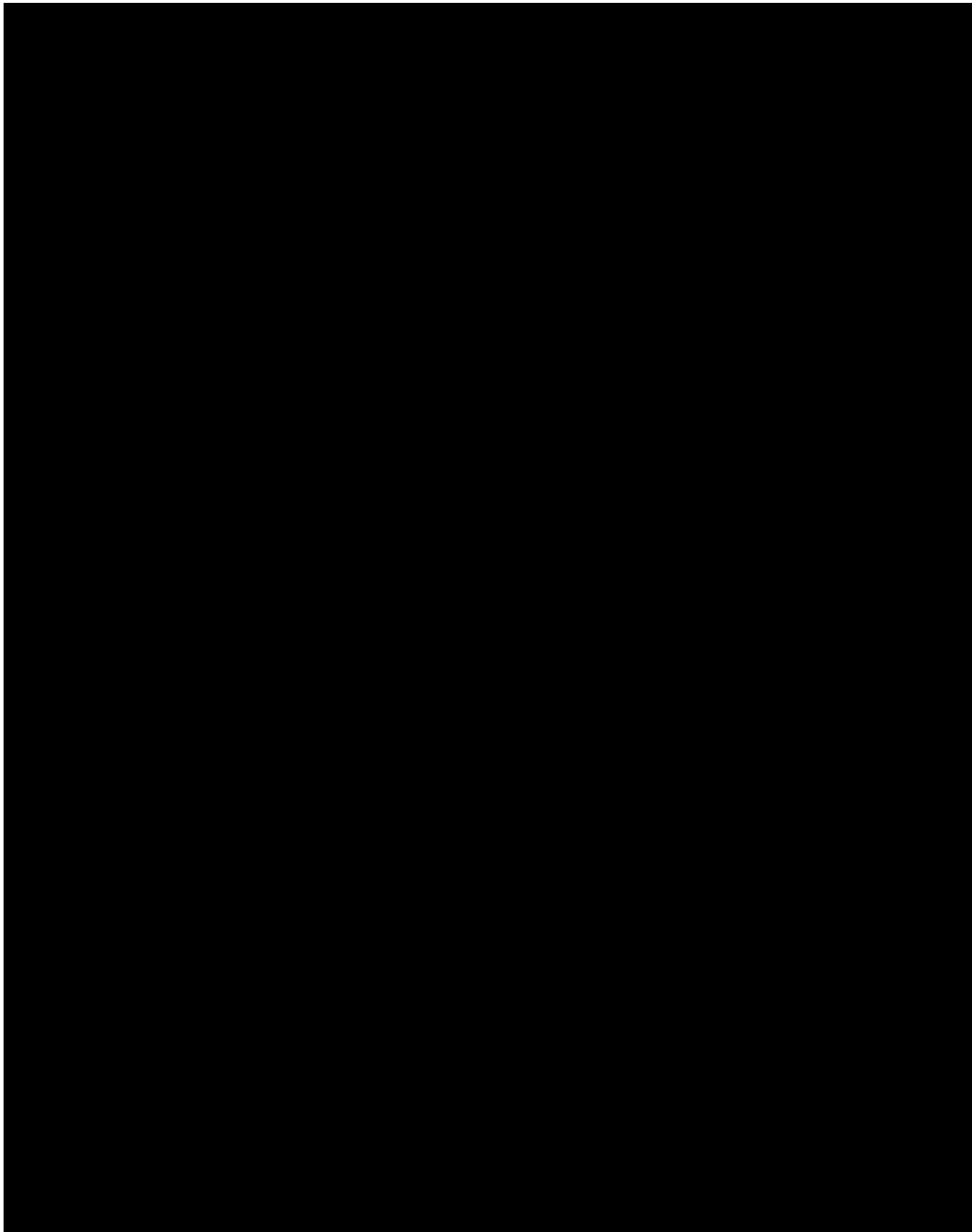
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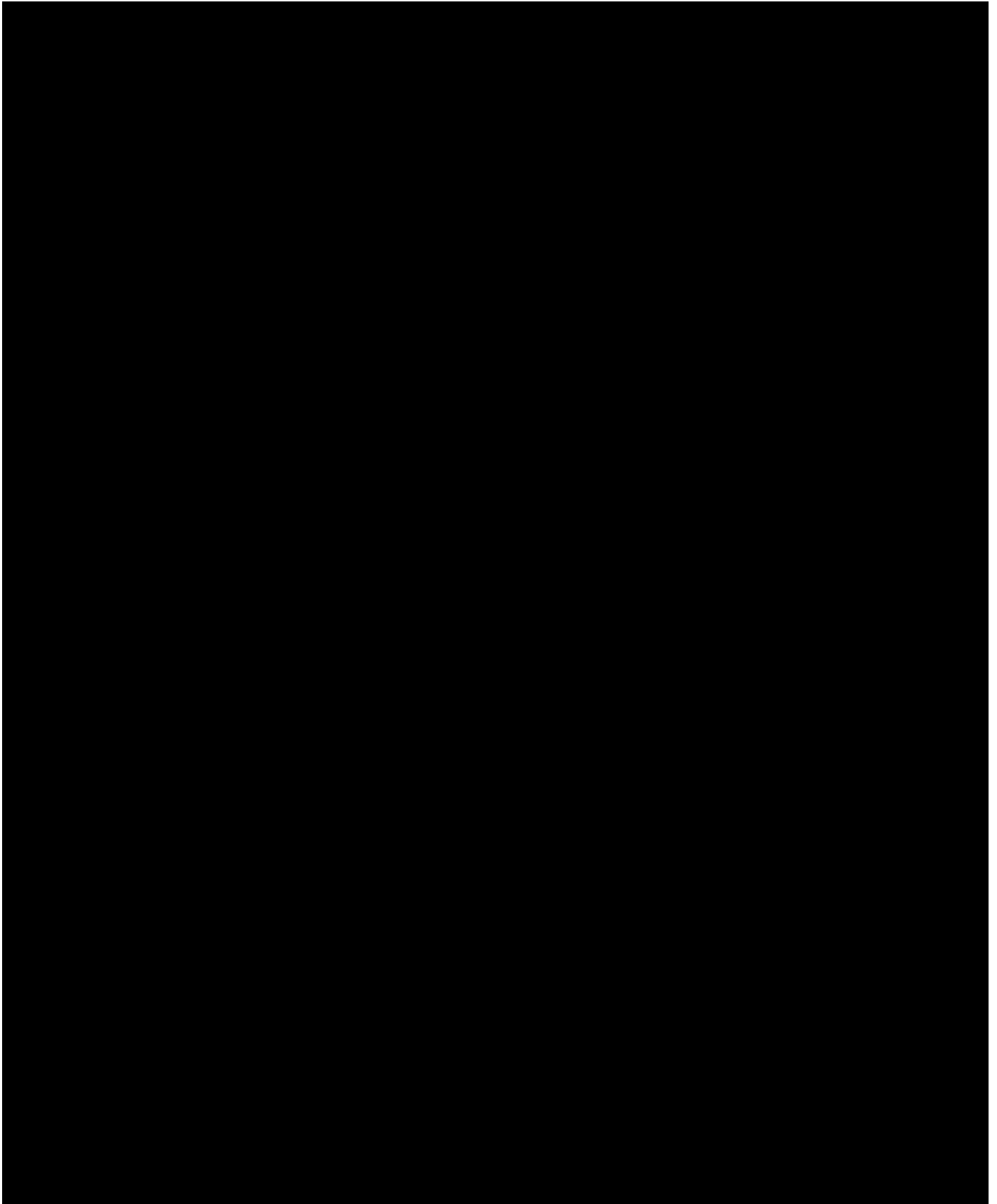
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6.2 Worker Exposure Information

7. Sites Not Controlled by the Submitter

7.1 Corn-based Ethanol Fermentation¹⁹³

7.1.1 Dry Mills Corn and Slurry

Whole kernel No. 2 yellow-dent corn is the most typical grain used for ethanol production though other grains such as milo or wheat may also be used usually in combination with corn. The grain is first milled to flour by a hammer mill to a particle size less than 2 mm. The corn flour is fed to a slurry mixer where it is mixed with water and recycled process water (backset) to form 30-33% solids slurry.

¹⁹³ Source: National Corn to Ethanol Research Center: www.ethanolresearch.com/pdf/Corn-to-Ethanol_Process_-_sab.pptx

7.1.3 Fermentation

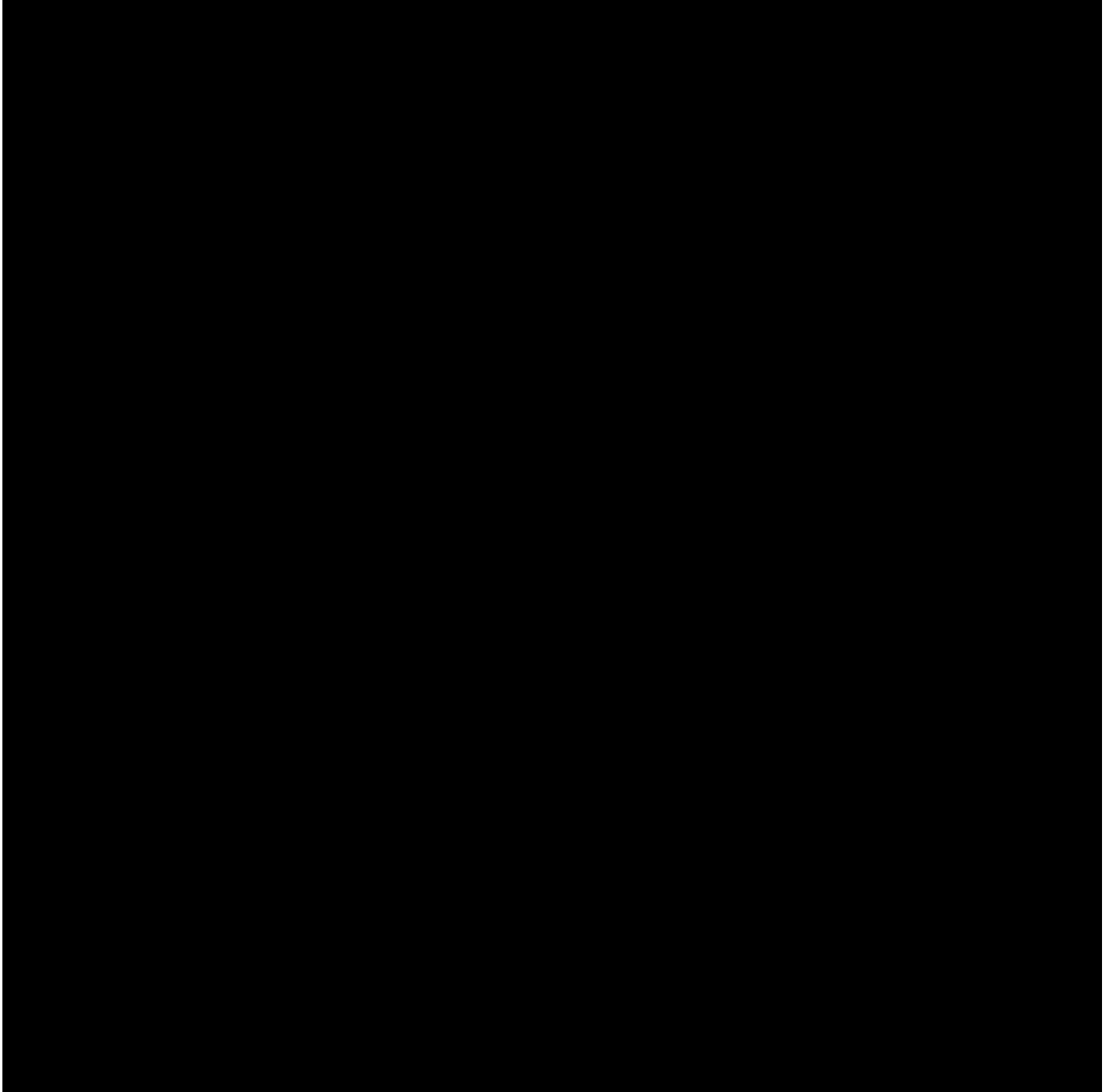
7.1.4 Distillation

Following fermentation, the whole broth, which is known as “beer,” is sent to distillation where, in the first stage or “beer stripper,” the ethanol/water mixture is evaporated from the residual solids including yeast cells, corn protein, corn oil and fiber. Ethanol along with water comes out through the top of the column, and the solid material including yeast, corn protein and fiber comes out of the bottom of the column also with water.

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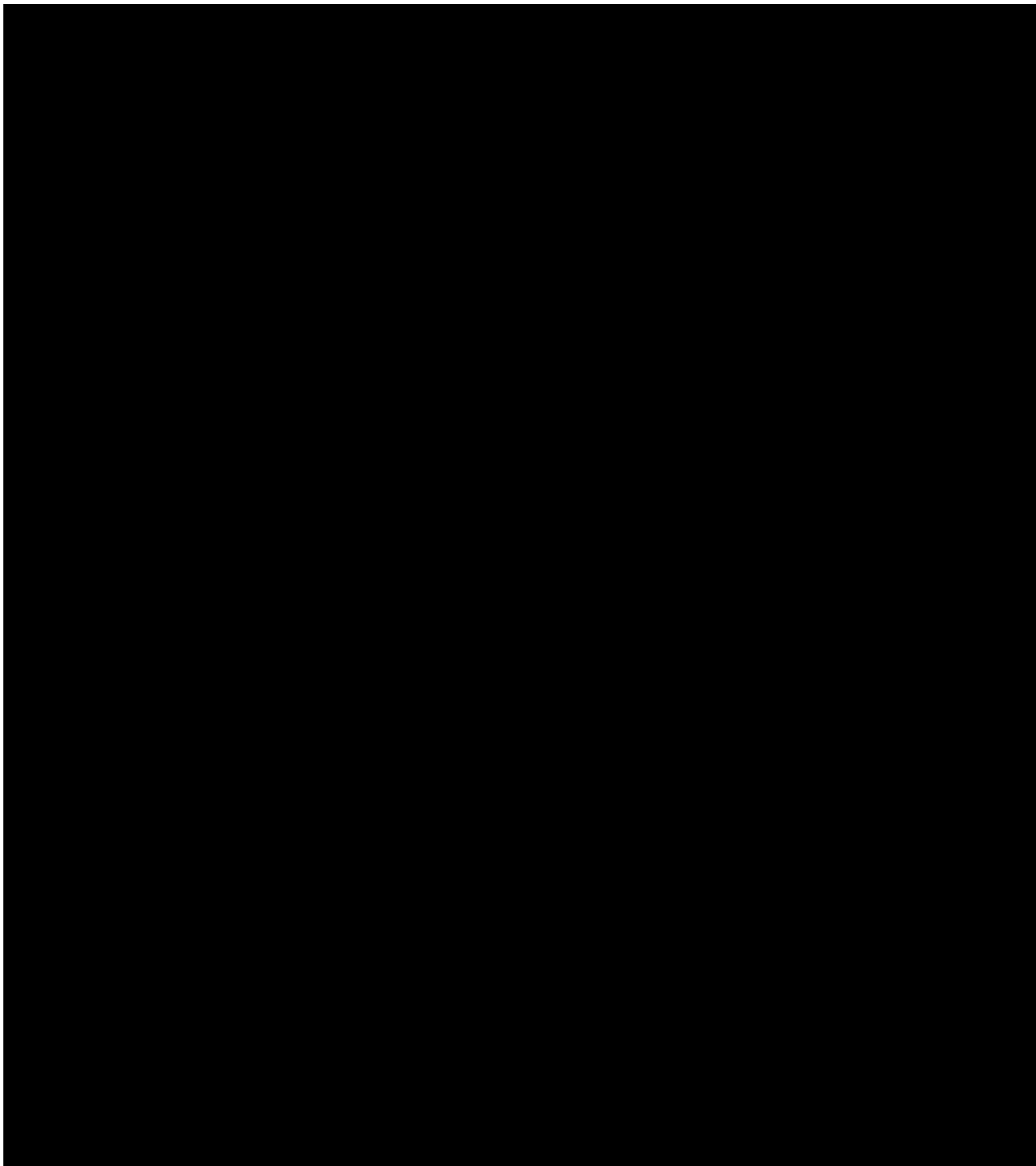


7.2 Worker Exposure Information

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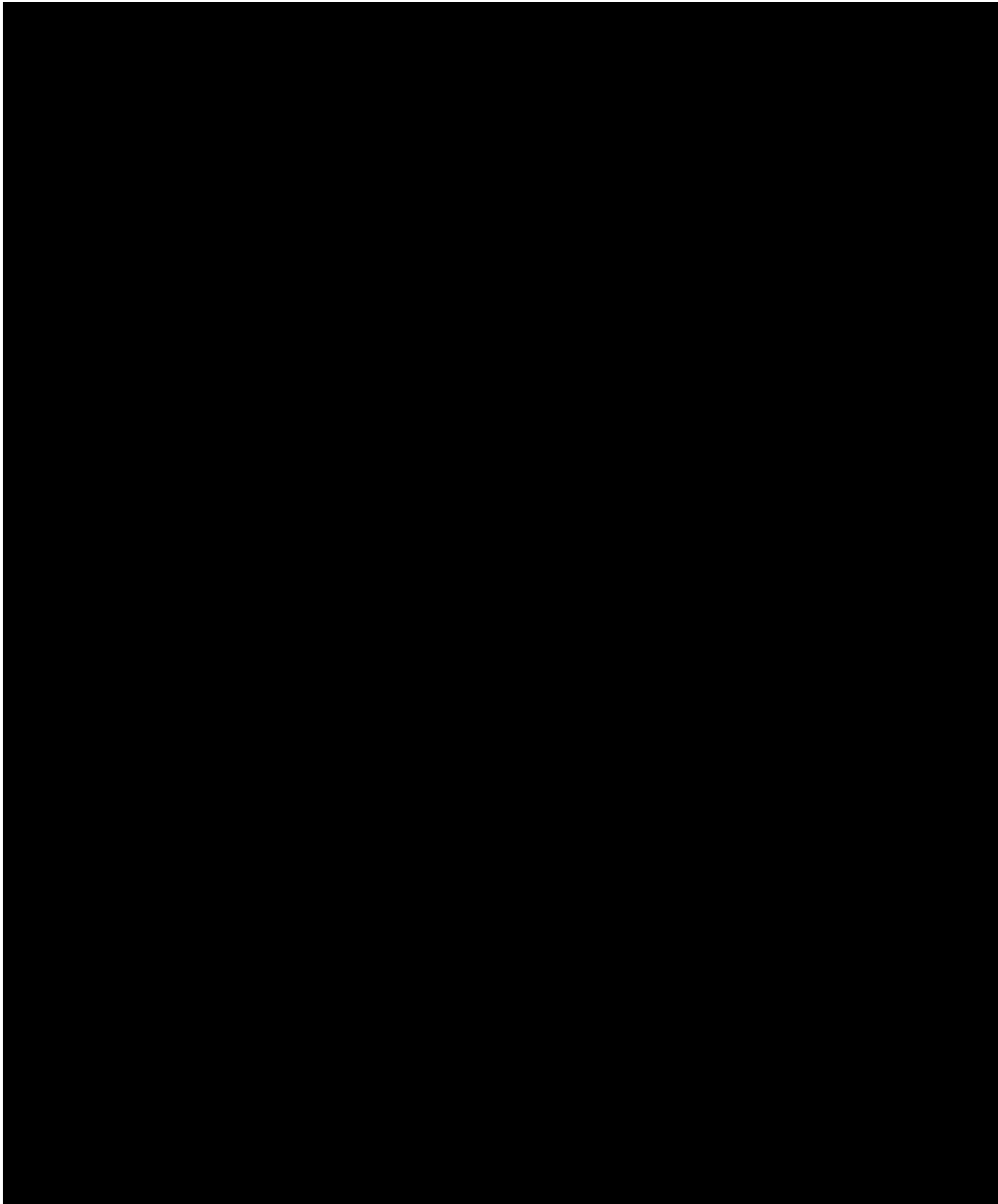
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8. Environmental Release

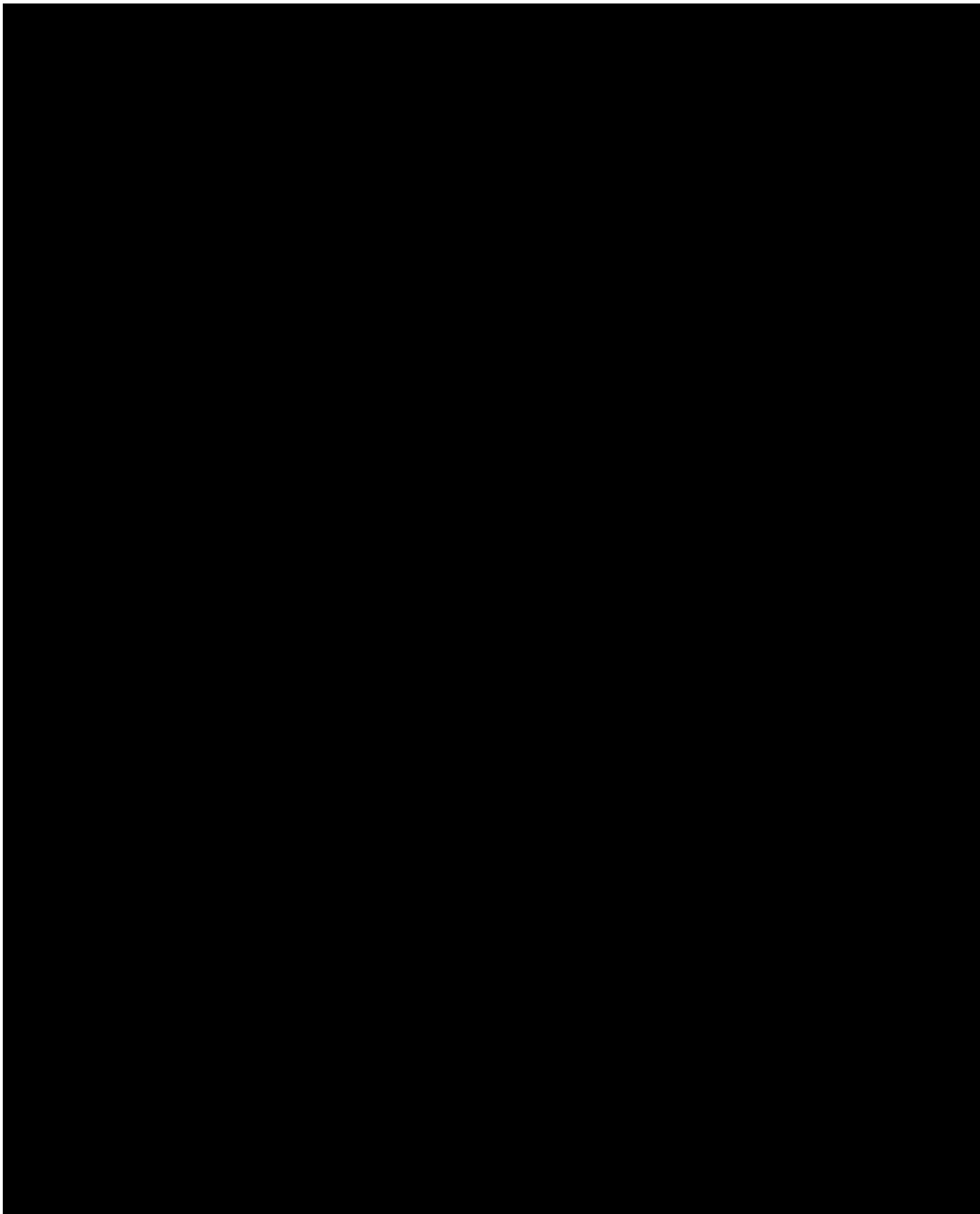
The EPA 1997 Final Risk Assessment for *S. cerevisiae* concludes (p. 11) that: “Releases of this microorganism to the environment through fermentation uses would not pose any significant ecological hazards, because this microorganism is ubiquitous in the environment and it is not pathogenic to animals or plants.”

8.1 Inactivation Information—Laboratory Scale

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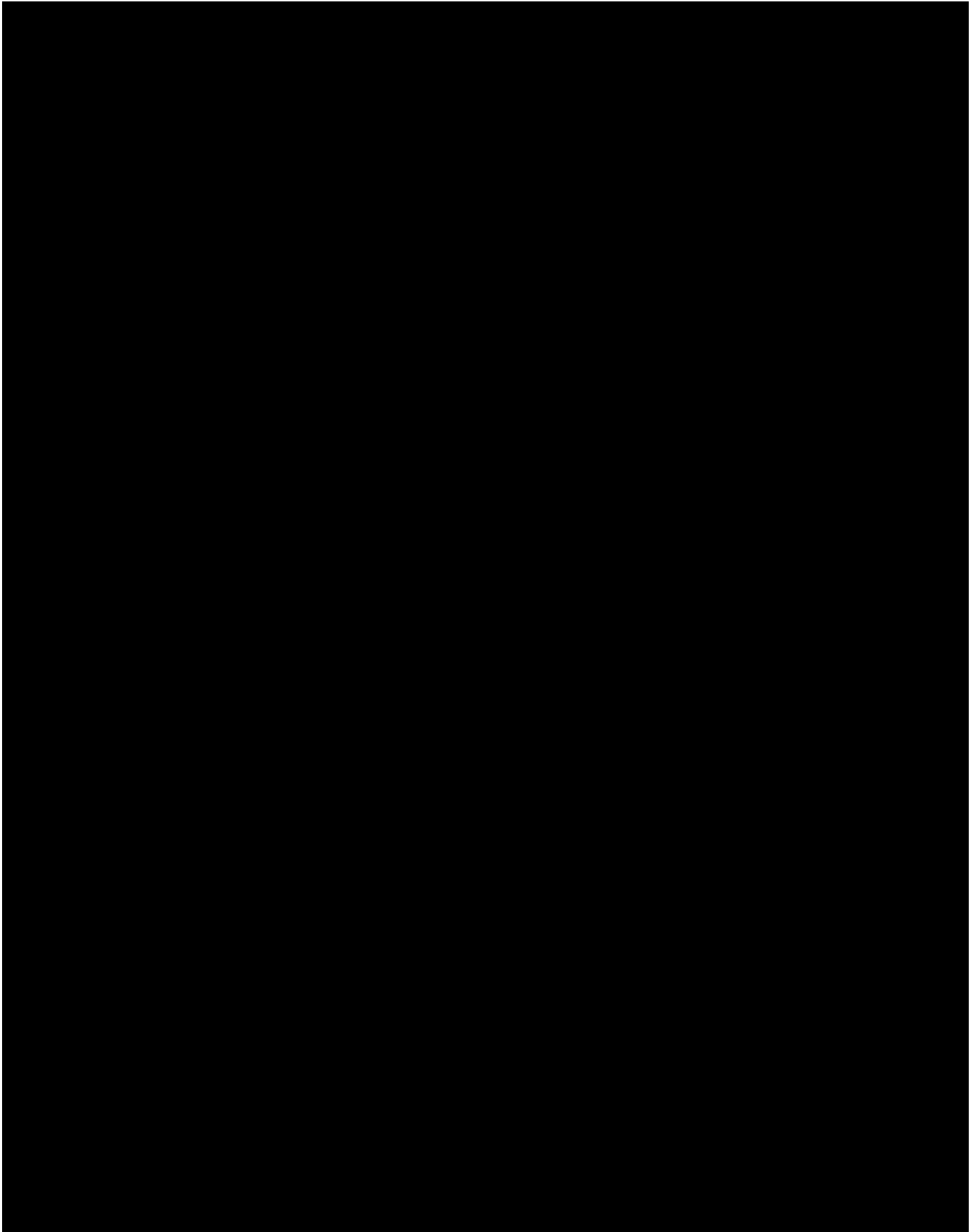
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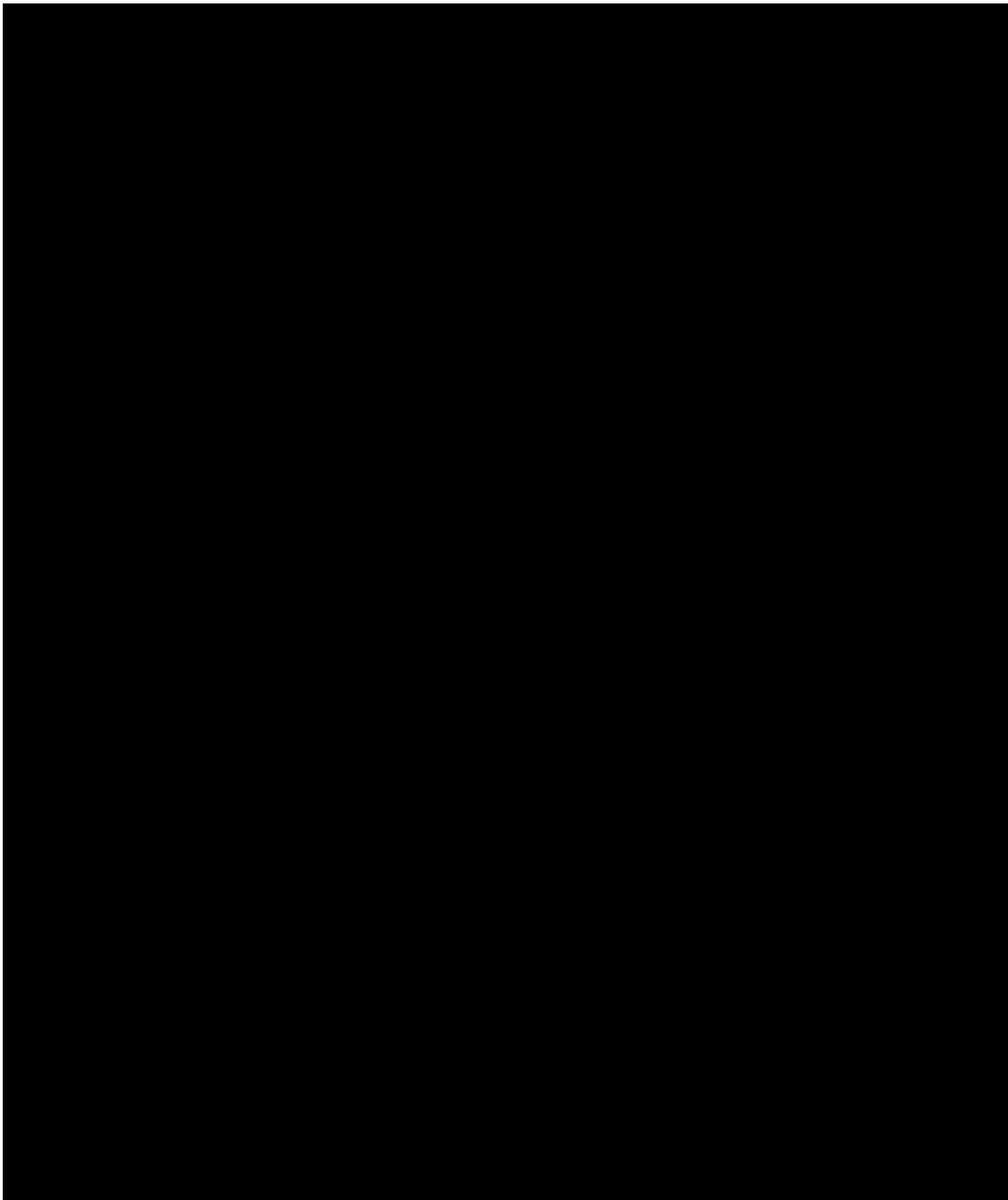
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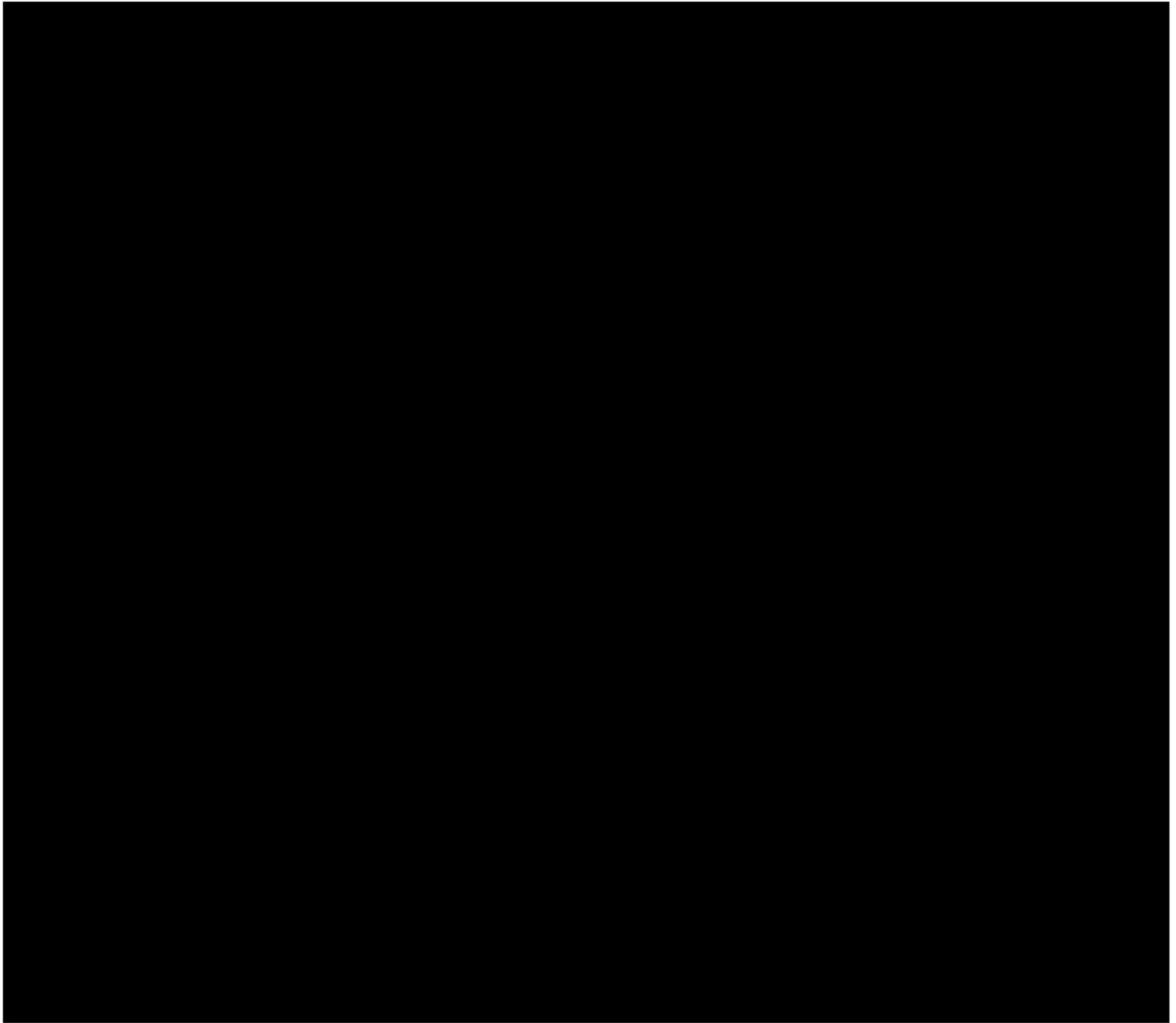
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8.3 Air Release Estimates—Yeast Production



8.4 Air Release Estimates—Ethanol Facilities

Air releases from inactivated modified yeast are low risk.

[Redacted text block containing multiple lines of information, likely a table or list of data points related to air release estimates.]

Under the Tier 1 exemption for modified *S. cerevisiae*, EPA considered a 2-log reduction target as appropriately protective. EPA states in its Rule on Microbial Products of Biotechnology: Summary of the Public's Comments and the Agency's Response that "[i]n the proposal EPA indicated that a 2-log reduction in viable microorganisms per cubic foot of air between the headspace and the actual vent port was the appropriate standard [for the Tier I exemption]" and characterized its position further as follows (U.S. EPA, 1997a):

EPA believes that it should allow some flexibility in the type of features manufacturers employ to minimize microbial releases as aerosols. A variety of fermenter equipment or features are commonly used by the industry such as demisters, wet scrubbers, cyclone separators, coalescing filters, and HEPA filters. These types of equipment reduce the number of microorganisms vented through exhaust gases from the fermenter. Moreover, as stated in the preamble (59 FR 45549), even if microorganisms are exhausted from the fermenter, their survival is likely to be limited due to the stress conditions of aerosolization, including shear forces, desiccation, and UV light exposure. Given the

comments received on the feasibility of this requirement and the variety of methods used by PMN submitters to reduce microbial numbers in aerosols, EPA believes that a specific numerical performance standard is less appropriate for inactivation of aerosols than it is for inactivation of liquid and solid wastes. EPA agrees with commenters who asserted that the majority of microorganisms potentially released from the fermentation facility would be found in the liquid and solid wastes.

Further, in its 1997 Final Risk Assessment for *S. cerevisiae* (U.S. EPA, 1997b), with respect to the use of engineering controls, EPA reviewed information submitted on physical containment and control technologies in the premanufacture notifications (PMNs) it had received for intergeneric microorganisms between 1986 and 1995. The following finding is relevant to this assessment:

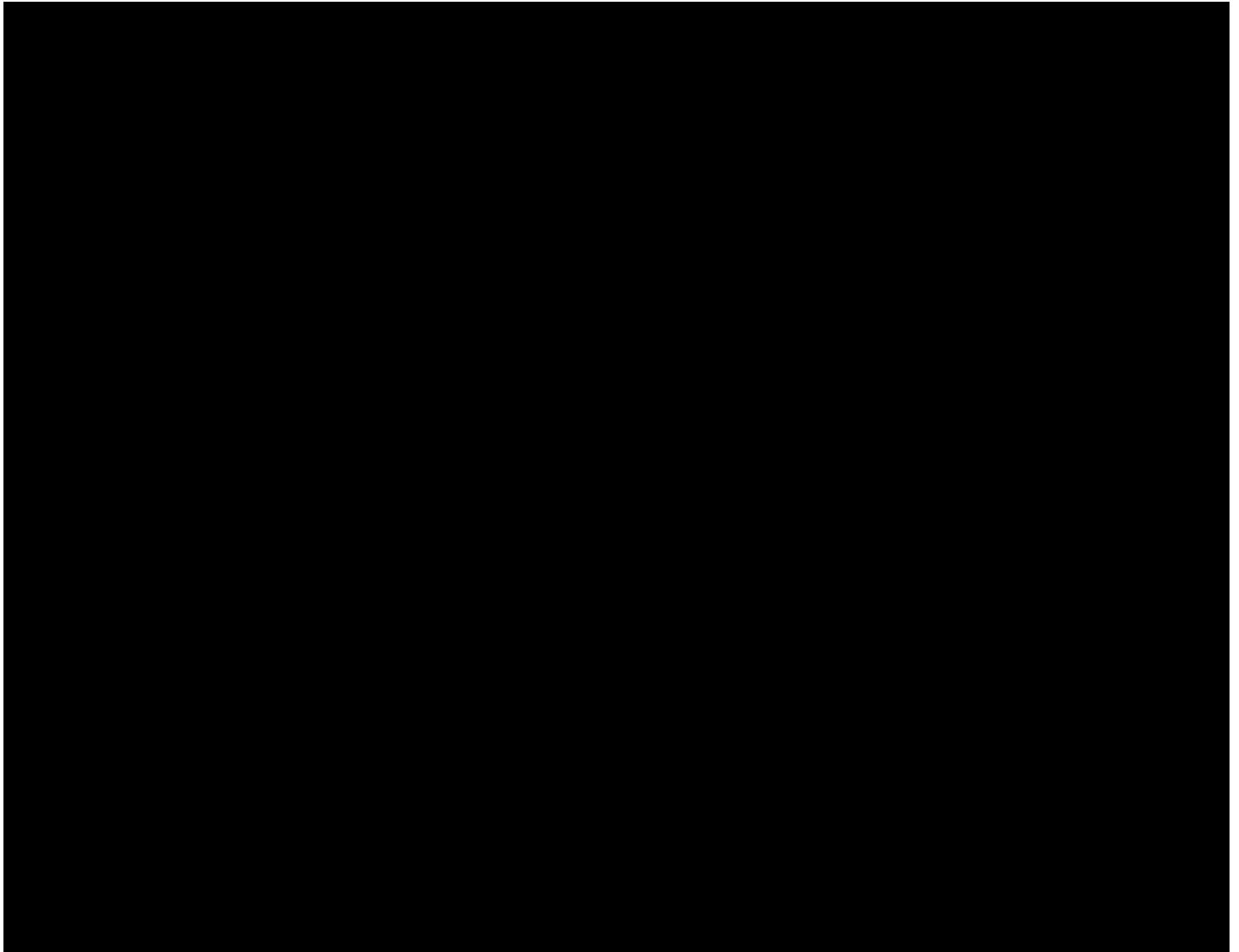
Examination of these PMNs revealed that the number of microorganisms potentially released through fermenter exhaust gases is negligible compared to the number contained in the liquid and solid waste streams. Even under a worst-case scenario of an uncontrolled release, as evaluated in the accompanying risk assessment, the number of viable microorganisms aerosolized with the fermenter exhaust gases would still be low, and therefore, the risk would remain low. Moreover, the use of a criterion requiring controls to minimize microbial numbers released through aerosolization at § 725.422, as compared to the worst-case scenario of an uncontrolled release, would result in lesser exposure, and therefore, lower risk than under the uncontrolled release scenario.

Uncontrolled releases are not standard industry practice because there are a number of economic considerations driving the control of exhaust gases such as maintaining proper molarity of the fermentation broth by the use of a vapor recovery system, maintaining sterility, and preventing release of microorganisms for proprietary reasons. Therefore, upon re-evaluation, the Agency decided that language requiring minimization of microbial concentrations in aerosols could be substituted for the requirement of the 2-log reduction performance criterion without affecting the no unreasonable risk finding necessary for a 5(h)(4) exemption under TSCA. The potentially increased exposure to this

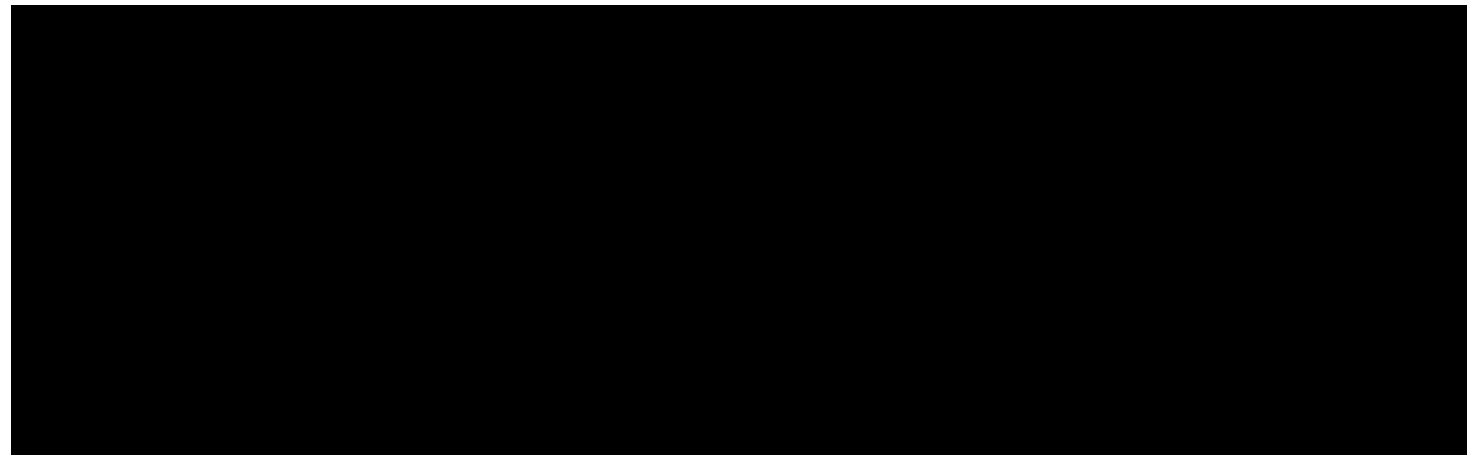
organism from the modification of the containment criteria from the proposed 2-log reduction to minimizing microbial numbers in exhaust gases does not change the risk of using this microorganism for fermentation.

EPA has identified air emission sources in ethanol fermentation facilities as including fermenter vents, openings, seals, and fittings, emergency relief valves, samples operations, rotary drum filters, and storage tank vents. Rotary drum filters are also a source of air emissions, estimated to have emissions of an additional 250 CFU/day (**Attachment 9**).

8.5 Water Release Estimate—Yeast Production Facilities



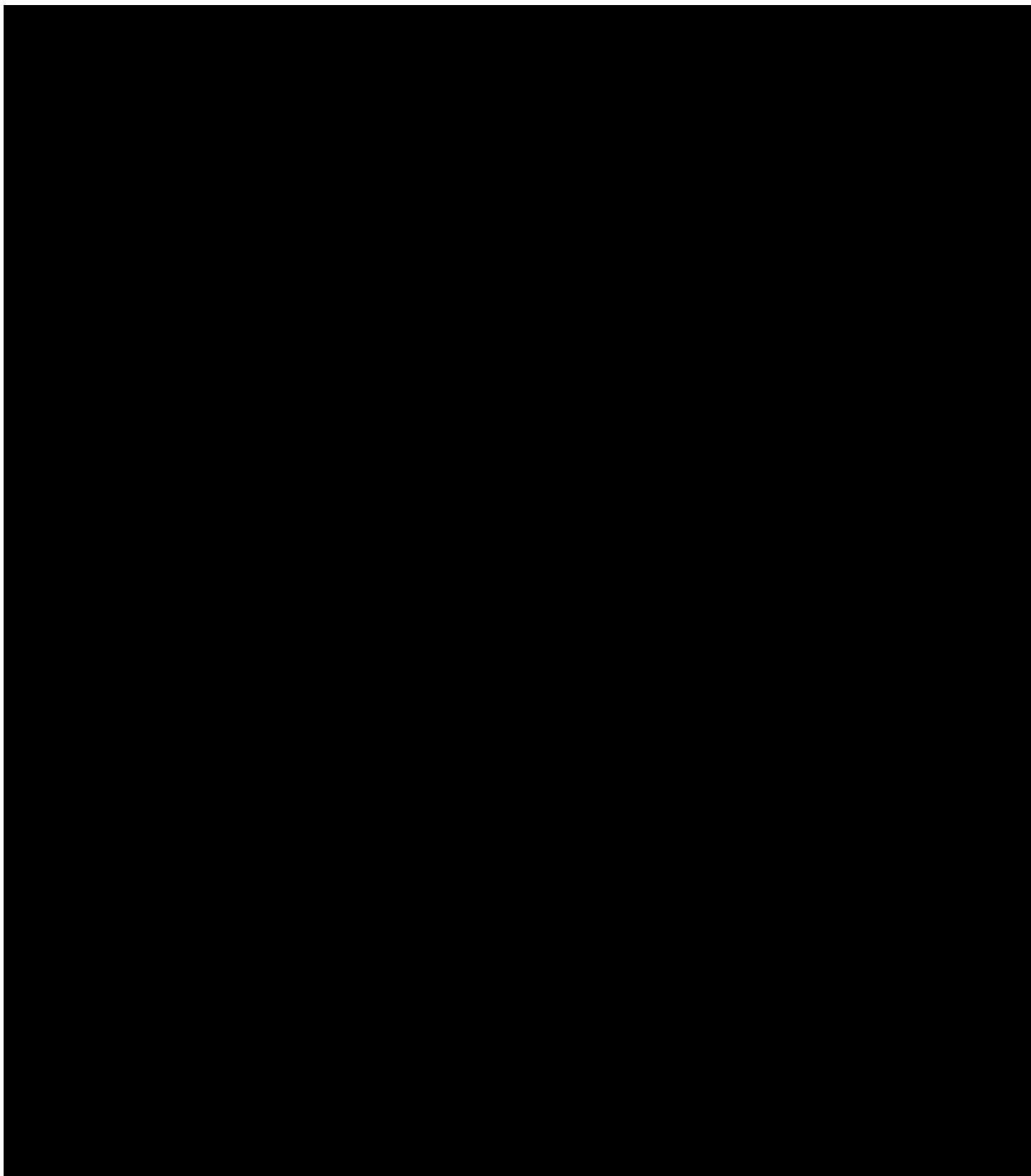
8.6 Water Release Estimates—Ethanol Facilities



[REDACTED]

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8.7 Solid Waste—Yeast Production Facilities

There are no significant anticipated releases of solids from yeast production facilities, as the solids produced in the facilities are the desired product (yeast biomass) that is shipped to customers and fuel ethanol facilities.

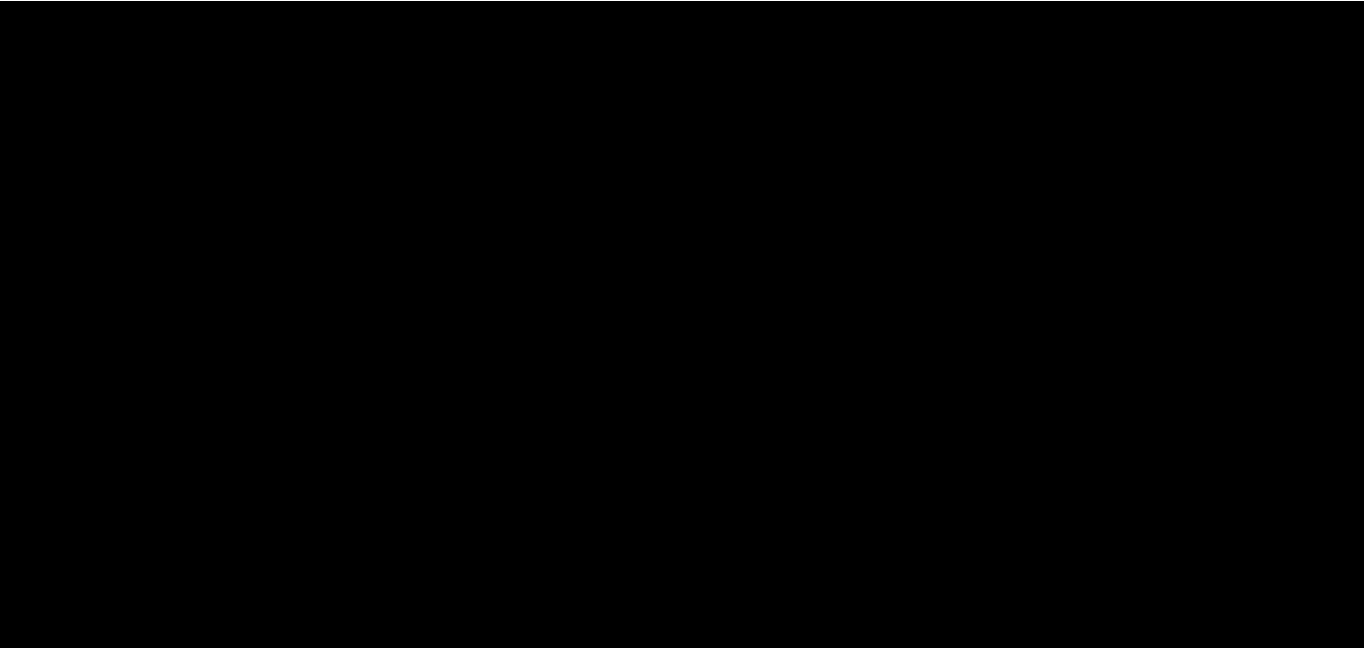
8.8 Solid Waste—Ethanol Production Facilities

The EPA's review concluded that solid waste is expected from disposal of the filter cake, which is typically sent to landfill, or spread onto land. EPA estimates that these solid releases are expected to contain inactivated cells on the order of 7×10^{15} cfu/day (**Attachment 9**).

8.9 Procedures for Disposal of Articles, Waste, Clothing, and Other Equipment

8.9 1 Laboratory

8.9.2 Ethanol Facility



9. Emergency Procedures

9.1 Yeast Production Facilities



9.2 Ethanol Production Facilities

Ethanol production facilities will typically be subject to state and federal requirements to have procedures in place that provide appropriate hazard and emergency preparedness measures.

Aspects of these measures may include the following: emergency classification system, government response, incident command, and evacuation/accountability. On-site emergency procedures will call for containment, deactivation (through use of bleach), proper disposal, and the use of personal protective equipment. In addition, facilities may have trained HazMat Technicians on-site to evaluate and respond to process upsets.

[REDACTED]

10. Health and Safety Data

[REDACTED]

11. Summary

[REDACTED]

Collectively, these conditions support the position that the microorganism contained in this MCAN does not pose any potential hazard nor does it cause any potential environmental impact any differently than other yeast strain found in nature or modified strains used in food, feed, pharmaceutical, or ethanol industries.